***Thermaerobacter marianensis* gen. nov., sp. nov., an aerobic extremely thermophilic marine bacterium from the 11 000 m deep Mariana Trench**

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A novel extremely thermophilic bacterium was isolated from the world's deepest sea-floor, the Mariana Trench Challenger Deep at a depth of 10897 m. Cells were Gram-reaction variable, non-spore-forming and non-motile rods without flagella. Growth was observed between 50 and 80 °C (optimum: 74–76 °C; 90 min doubling time), pH 5.4 and 9.5 (optimum: pH 7.0–7.5) and 0.5 and 5% sea salts (optimum: 2% sea salts). The isolate was a strictly aerobic heterotroph capable of utilizing as sole energy and carbon source: yeast extract, peptone, cellulose, starch, chitin, casein, Casamino acids, a variety of sugars, carboxylic acids and amino acids. The G+C content of the genomic DNA was 72.5 mol%. Phylogenetic analysis based on 16S rRNA sequences placed this aerobic, high-G+C-content bacterium among the members of the Gram-positive, low-G+C-content anaerobic thermophilic bacteria within the *Bacillus*–*Clostridium* subphylum. On the basis of the physiological and molecular properties of the new isolate, the name *Thermaerobacter marianensis* gen. nov., sp. nov. (type strain 7p75a^T = JCM 10246^T) is proposed.

Keywords: *Thermaerobacter marianensis* gen. nov., sp. nov., thermophile, deep-sea micro-organism

INTRODUCTION

Recently, an increasing number of hyperthermophilic micro-organisms, capable of growing at temperatures up to 90 °C, have been isolated from deep-sea hydrothermal environments (Gonzalez *et al.*, 1995; Godfroy *et al.*, 1996, 1997; Antoine *et al.*, 1997; Blöchl *et al.*, 1997; Canganella *et al.*, 1997b; Huber *et al.*, 1997). Most of them are new members of *Thermococcales* and *Thermotogales*, which are anaerobic, heterotrophic and hyperthermophilic members of the domains *Archaea* and *Bacteria*, respectively (Gonzalez *et al.*, 1995; Godfroy *et al.*, 1996, 1997; Antoine *et al.*, 1997; Canganella *et al.*, 1997a), but some are phylogenetically and physiologically distinct hyperthermophiles (Blöchl *et al.*, 1997; Huber *et al.*, 1997). To date, however, the number of reports of cultivation and isolation of deep-sea thermophilic micro-organisms

that grow optimally at somewhat lower temperatures between 60 and 80 °C have been few (Martinson *et al.*, 1995; Harmsen *et al.*, 1997) and the distribution and diversity of such bacteria are still unclear.

From a mud sample of the Challenger Deep in the Mariana Trench at the depth of 10897 m, Takami *et al.* (1997) isolated a number of heterotrophic bacteria under various culture conditions. These isolates included not only psychrophilic and mesophilic bacteria but also several new phylogenetic lineages of thermophiles capable of growth at temperatures between 55 and 75 °C within the Gram-positive, low-G+C-content group of bacteria (Takami *et al.*, 1997). This observation implies that the extremely deep marine environments with high hydrostatic pressures and low temperatures harbour a diverse group of thermophiles and exploration of these environments may lead to undiscovered thermophilic micro-organisms.

In this study, we succeeded in the cultivation and isolation of a novel strictly aerobic extremely thermo-

The DDBJ accession number for the 16S rDNA sequence of 7p75a^T is AB011495.

philic bacterium optimally growing at 75 °C from a mud sample obtained from the Challenger Deep in the Mariana Trench at a depth of 10897 m. We describe the characterization of this new isolate and propose the new genus name *Thermaerobacter*.

METHODS

Sample collection. A mud sample was obtained from the bottom of the Challenger Deep (11° 21' 11" N, 142° 25' 49" E) at a depth of 10897 m by means of the unmanned submersible Kaiko in a dive performed on 2 March 1996, as described previously (Takami *et al.*, 1997). The sample was collected in a sterile 50 ml Falcon tube, which was tightly inserted in a tube holder, brought to the sea surface without microbial contamination from other depths, and immediately frozen at -85 °C (Kato *et al.*, 1997; Takami *et al.*, 1997). The sample was stored at -85 °C prior to incubation.

Enrichment and purification. The thawed sample was used to inoculate a series of media including modified MJ medium (Sako *et al.*, 1996) and MJP medium, described below, and the cultures were incubated at 70, 76, 82, 88 or 95 °C. All tubes of MJP medium inoculated with the mud sample became turbid after 2 d incubation at 76 °C. To obtain a pure culture, the dilution-to-extinction technique was employed (Baross, 1995). After the cell density of the enrichment culture reached approximately 10^8 cells ml⁻¹, 10 separate dilution series were prepared in which five 1:10 dilutions of the culture were followed by 20 1:2 dilutions. Each dilution was performed using MJP medium, and the cultures were then incubated for at least 3 d at 76 °C. The culture in the tube showing growth at the highest dilution was designated strain 7p75a^T.

Culture medium and conditions. The new isolate was routinely cultivated in modified MJ medium (Sako *et al.*, 1996), MJP medium which contained 1 g trypticase peptone per l of MJ synthetic seawater. MJ synthetic seawater was composed of NaCl, 30.0 g; K₂HPO₄, 0.14 g; CaCl₂·2H₂O, 0.14 g; NH₄Cl, 0.125 g; Na₂NO₃, 0.125 g; MgSO₄·7H₂O, 3.4 g; MgCl₂·6H₂O, 4.18 g; KCl, 0.33 g; NiCl₂·6H₂O, 0.5 mg; Na₂SeO₃·5H₂O, 0.5 mg; Fe(NH₄)₂(SO₄)₂·6H₂O, 0.01 g; trace mineral solution 10 ml, consisting of (l⁻¹) nitrilotriacetic acid (1.5 g), MgSO₄·7H₂O (3.0 g), MnSO₄·2H₂O (0.5 g), NaCl (1.0 g), FeSO₄·7H₂O (0.1 g), CoSO₄·7H₂O (0.18 g), CaCl₂·2H₂O (0.1 g), ZnSO₄·7H₂O (0.18 g), CuSO₄·5H₂O (0.01 g), KAl(SO₄)₂·12H₂O (0.02 g), H₃BO₃ (0.01 g), Na₂MoO₄·2H₂O (0.01 g), NiCl₂·6H₂O (0.025 g), Na₂SeO₃·5H₂O (0.3 mg); the pH of trace mineral solution was adjusted to 7.0 with NaOH. The pH of MJP medium was adjusted to 7.2–7.4 with NaOH at room temperature, unless otherwise noted. For testing the effects of pH on growth, the pH of MJP medium containing 20 mM of sodium succinate (pH 5.0), MES (pH 5.5–6.5), HEPES (pH 7.0–8.0) or BTP {1, 3-bis[tris(hydroxymethyl) methylamino] propane} (pH 8.5 or above) was adjusted to various values with H₂SO₄ or NaOH at room temperature. For testing the effects of sea salts concentration on growth, varying dilutions of 2 × MJ synthetic seawater containing 0.1 % (w/v) peptone (pH 7.2 adjusted with NaOH at room temperature) was used instead of MJP medium. To examine the utilization of carbon-containing substrates, the peptone in MJP medium was replaced with various concentrations of substrates. Microaerobic or anaerobic growth of the new isolate was tested as described by Huber *et al.* (1992) or Balch & Wolfe (1976), respectively. In these experiments, 20 ml medium

was dispensed into a 100 ml glass bottle (Schott Glaswerke) which was tightly sealed with a butyl rubber stopper and the gas phase was exchanged with either H₂/CO₂/N₂/O₂ (60:10:25:5, 200 kPa) or N₂/CO₂/O₂ (79:20:1, 200 kPa) for microaerobic conditions, and with either H₂/CO₂ (80:20, 200 kPa) or N₂ (100 %, 200 kPa) for anaerobic conditions. In order to insure anaerobic growth condition, either sterilized 5 % (w/v) Na₂S·9H₂O or cysteine·HCl solution (pH 7.2, adjusted with H₂SO₄ or NaOH) were added in the medium to the final concentration of 0.05 % (w/v). The state of oxygenation was monitored using resazurin [1 mg (l medium)⁻¹] and the cultures were incubated at 75 °C.

Determination of growth. Growth of the new isolate was determined by direct cell count after staining with 4',6'-diamidino-2-phenylindole (DAPI) (Porter & Feig, 1980) using a Nikon Optishot microscope equipped with a Nikon FX-II camera system. Cultures prepared in duplicate were grown in 300 ml flasks containing 100 ml medium in a temperature-controlled dry oven (Taitec) and were shaken at 100 r.p.m. in all cases. The pH growth curve was determined at 75 °C and the sea salts growth curve was determined at 75 °C and pH 7.2.

Light and electron microscopy. Cells were routinely observed with a phase-contrast microscope (Optishot 2; Nikon). For microscopy at 75 °C, a drop of culture at 75 °C was placed on a slide preheated to 80 °C and observed immediately. Micrographs were obtained using a Nikon Optishot microscope equipped with a Nikon FX-II camera system.

Transmission electron microscopy of negatively stained cells was carried out as described by Zillig *et al.* (1990). Cells grown at 75 °C in the mid-exponential phase of growth were negatively stained with 2 % (w/v) uranyl acetate and observed under a JEOL JEM-1210 electron microscope at an accelerating voltage of 80 kV. For ultrathin sectioning, the rapid-freezing and substitution fixation method was used (Amako *et al.*, 1983). The thin section was stained with uranyl acetate and lead citrate and observed with a JEOL JEM-1210 electron microscope at an accelerating voltage of 80 kV. The scanning electron microscopy of the new isolate was carried out as described by Padilla *et al.* (1997) with minor modification. Cells grown at 75 °C in the mid-exponential phase of growth were collected on a 0.45 µm pore size Millipore HA filter and fixed with 2.5 % (v/v) glutaraldehyde in 50 mM Tris/HCl (pH 7.5) containing 3 % (w/v) NaCl at 75 °C for 30 min. After dehydration in a graded ethanol series, the specimen was immersed in t-butyl alcohol and dried using a freeze drying method. The specimen was observed using a JEOL JFC-1100E scanning microscope at an accelerating voltage of 5 kV.

Sporulation test. The spore-forming ability of the new isolate was examined by physiological induction under various growth conditions and using molecular determination of sporulation genes (Brill & Wiegell, 1997). Cells were cultivated to early exponential growth phase under optimal conditions and then exposed to lower (60, 55, 50 and 45 °C) or higher temperatures (80 and 85 °C). In other experiments, exponentially growing cells in MJP medium under optimal conditions were transferred into MJ seawater containing 0.05, 0.01, 0.005 or 0.001 % (w/v) peptone at various temperatures (45–85 °C). The exponentially growing cells in MJP medium under optimal conditions were also incubated on plates of MJP seawater containing a varying concentration of peptone (0.001–0.1 %) and 2, 2.5 or 3 % (w/v) agar (melting temperature, 80 °C; Nacalai Tesque) at various temperatures (45–85 °C). During the exposure and

cultivation, the cells were observed with a phase-contrast microscope.

The molecular determination of *spo0A* and *ssp* genes, which are involved in sporulation of many endospore-forming bacteria and have been used as molecular probes for the screening of spore-forming bacteria (Brown *et al.*, 1994; Setlow, 1988; Mohr *et al.*, 1991; Brill & Wiegel, 1997), was performed by PCR. The primers for amplification were designed and the PCR reactions were carried out as described by Brill & Wiegel (1997). All PCR products were directly sequenced on both strands by the dideoxynucleotide chain termination method using DNA sequencer model 373As (Perkin Elmer/Applied Biosystems). The nucleotide sequences of these products were analysed using Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) to estimate the degree of similarity to other known *spo0A* and *ssp* genes.

Effect of pressure on growth. The effect of hydrostatic pressure on growth was examined using a 'DEEP-BATH' system, a high-pressure/high-temperature bioreactor (Moriya *et al.*, 1995; Canganella *et al.*, 1997b). Cells pre-cultured in MJP medium at 75 °C were transferred to a 1.5 l glass vessel containing 1 l MJP medium which was vigorously agitated, pre-heated at 75 °C and pre-pressurized at 0.5, 20, 40 or 60 MPa under a gas phase of air. Samples were taken at regular intervals and the growth under each pressure condition was determined by direct cell count after staining with DAPI.

Organic substrates for growth. In an attempt to find organic substrates which could support growth of the isolate, experiments were conducted using MJ seawater containing potential substrates (pH 7.2). These were added at concentrations of 0.02 and 0.2 % (w/v). The cells were pre-cultured in each medium prior to inoculation of the same medium. These tests were run in duplicate at 75 °C.

Cellular fatty acid composition. Cellular fatty acid composition was analysed using cells of strain 7p75a^T cultivated in MJP medium at 75 °C. Cellular fatty acids were determined as described by Kawasumi *et al.* (1984).

Isolation and base composition of DNA. DNA was prepared as described by Marmur & Doty (1962) and Lauerer *et al.* (1986). The G + C content of DNA was determined by direct analysis of deoxyribonucleotides using HPLC (Tamaoka & Komagata, 1984). Calf thymus DNA (42 mol% G + C; Sigma) (Marmur & Doty, 1962) and non-methylated DNA from bacteriophage λ (49.8 mol% G + C; TaKaRa) (Sanger *et al.*, 1982) were used as reference materials.

Amplification of 16S rRNA gene and sequence determination. The 16S rRNA gene (rDNA) was amplified by the polymerase chain reaction (PCR) using Eubac 27F and 1492R primers (DeLong, 1992). The 1.5 kb PCR product was directly sequenced by the dideoxynucleotide chain termination method using a DNA sequencer model 373As (Perkin Elmer/Applied Biosystems). The rDNA sequence was analysed using SIMILARITY-RANK, ALIGN-SEQUENCE and data from the Ribosomal Database Project (RDP) (Larsen *et al.*, 1993) and BLAST was used to estimate the degree of similarity to other rDNA sequences.

Data analysis. The almost complete sequence (1488 bp) of 16S rDNA of 7p75a^T was manually aligned to SSU rDNA data from the RDP based on primary and secondary structure considerations using the Genetic Data Environ-

ment (GDE) multiple sequence editor. Phylogenetic analyses were restricted to nucleotide positions (1263 homologous positions) that were unambiguously alignable in all sequences. Least-squares distance matrix analysis (Olsen, 1988), based on evolutionary distances, was estimated by using the correction of Kimura (1980). Neighbour-joining analysis was accomplished using the ODEN software package (version 1.1, National Institute of Genetics, Mishima, Japan). Maximum-likelihood analysis was performed using fastDNAmI (version 1.0; distributed by RDP). Bootstrap analysis was used to provide confidence estimates for phylogenetic tree topologies.

RESULTS

Enrichment and purification

Enrichment from a mud sample in MJP medium occurred at 70 and 76 °C but not at higher temperatures of 82, 88 or 95 °C, and a population of long, thin, rod-shaped cells was observed after 2 d incubation. Although the rod-shaped cells could not form colonies on plates of MJP medium containing 2, 2.5 and 3 % (w/v) agar (melting temperature, 80 °C; Nacarai Tesque) and in the tubes of MJP medium containing various concentrations of agar (0.5–2 %, w/v) at temperatures of 50, 55, 60, 65 or 75 °C, they were successfully purified by the dilution-to-extinction technique in 10 separate series at 76 °C. The culture in the tube showing growth at the highest dilution was designated strain 7p75a^T (= JCM 10246^T; JCM, Japan Collection of Microorganisms, Wako, Japan). The purity of this culture was routinely confirmed by microscopic examinations and by partial sequencing of 16S rRNA gene using several PCR primers. All further experiments were performed with isolate 7p75a^T.

Morphology

The cells were thin straight to slightly curved rods, 2–7 μ m long and 0.3–0.6 μ m wide during the exponential phase of growth (Fig. 1a, c), and stained weakly Gram-positive, but stained Gram-negatively and became very long (more than 10 μ m) in the stationary phase of growth (Fig. 1b). They often appeared singly but pairs were also observed (Fig. 1a, c and d). As observed by the light microscopy at 76 °C, the cells were non-motile, and the results of transmission electron microscopy of negatively stained cells indicated that these cells had no flagella (Fig. 1d). Thin section electron micrographs showed the presence of a thin peptidoglycan layer (Fig. 1e and 1f), which was consistent with the weak Gram staining observed.

No spore formation by physiological induction was observed in any phase of growth under any of the conditions. In the PCR determination of sporulation genes, the targeted segments of *spo0A* were consistently amplified under a range conditions as described by Brill & Wiegel (1997) (data not shown). However, no nucleotide sequence having similarity to other known

Fig. 1. Light and electron micrographs of *Thermoaerobacter marianensis* cells. (a) Phase-contrast micrograph of exponentially growing cells; bar, 10 µm. (b) Phase-contrast micrograph of cells in the stationary phase; bar, 10 µm. (c) Scanning electron micrograph of exponentially growing cells occurring singly and in pairs; bar, 1 µm. (d) Electron micrograph of cells in pairs negatively stained; bar, 1 µm. (e) (f) Electron micrograph of a thin section of an exponentially growing cell. Arrow indicates the putative peptidoglycan layer; bars, 0.5 µm (e) and 0.1 µm (f), respectively.

ssp gene sequences was obtained. On the basis of these negative results for sporulation, the new isolate appeared to be a non-spore-forming bacterium.

Physiological characterization of growth

The new isolate grew only under aerobic culture conditions, indicating that isolate 7p75a^T was a strictly aerobic bacterium. Under both microaerobic and strictly anaerobic conditions, no growth occurred.

The isolate grew over the temperature range of about 50–80 °C, showing an optimum growth at 74–76 °C and the generation time at these temperatures was about 90 min at pH 7.2–7.4 (Fig. 2a). No growth was observed at 82 °C. Growth of the new isolate at 75 °C occurred between pH 5.5 and 9.5, with optimum growth about 7.5 (Fig. 2b). No growth was detected below pH 5.5 or above pH 9.5.

Strain 7p75a^T required sea salts for growth. It grew over the concentration range of about 0.5–5% sea salts, with optimum growth at around 2% sea salts at 75 °C and pH 7.2–7.4 (Fig. 2c). Below 0.5% or above

5% sea salts, growth was inhibited but the cells did not lyse.

The isolate was sensitive to hydrostatic pressure despite a high *in situ*-hydrostatic pressure (about 110 MPa) of the isolation site. In the 'DEEP-BATH' system (Moriya *et al.*, 1995), it grew slowly with a generation time of about 6 h and reached a maximum cell density of 4×10^7 cells ml⁻¹ at 75 °C at 0.5 MPa. Under higher hydrostatic pressures of 20, 40 and 60 MPa, no growth occurred.

Nutrition

The isolate was a heterotroph. In a medium containing 0.2% (w/v) peptone and 0.2% (w/v) yeast extract, 7p75a^T grew vigorously with a generation time of about 90 min and reached a maximum cell density of 6×10^8 cells ml⁻¹ at 75 °C. As shown in Table 1, somewhat lower maximum cell densities were obtained with a variety of sugars, organic acids and amino acids as the sole energy and carbon source. The new isolate was weakly capable of utilizing tartrate, succinate, lactate, acetate, valine, proline, serine, lysine, arginine

Fig. 2. Effects of temperature (a), pH (b) and sea salts concentration (c) on growth of *T. marianensis*. (a) Growth was determined in MJ medium at pH 7.2–7.4. No growth occurred below 50 °C and above 80 °C. (b) Growth was determined in MJ medium with various buffers at 75 °C. The pH of the medium containing appropriate buffer systems was adjusted with HCl or NaOH at room temperature. No growth occurred below pH 5.5 or above pH 9.5. (c) Growth was determined in varying dilutions of 2 × MJ synthetic seawater containing 0.1% (w/v) peptone at 75 °C and pH 7.2–7.4. No growth was observed at 0 or 6% sea salts concentration.

or histidine as a substrate for growth (Table 1). Very weak growth took place with cellulose, glycine, alanine, leucine, methionine or phenylalanine, as the substrate, and no growth occurred with lignin, fructose, tryptophan or tyrosine as the substrate (Table 1). In order to determine the substantial cellulose utilization of the isolate, the extracellular cellulolytic activity was examined based on the carboxymethyl-cellulase (CMCase) assay (Wood *et al.*, 1988). The CMCase activity was detected in the supernatant of

Table 1. Substrate utilization by *Thermaerobacter marianensis*

+++ , $> 5 \times 10^8$ cells (ml culture)⁻¹ (final concentration); ++ , 8×10^7 – 5×10^8 cells (ml culture)⁻¹ (final concentration); + , 3×10^7 – 8×10^7 cells (ml culture)⁻¹ (final concentration); –, no growth. For substrates at both 0.2 and 0.02%, results were ++ for peptone, yeast, extract, starch, xylan, chitin, maltotriose, cellobiose, lactose, trehalose, saccharose, tartrate, succinate, propionate, 2-aminobutyric acid, malate, lactate, pyruvate, acetate, glycerol, cysteine, proline, glutamine, asparagine, aspartate, glutamate, lysine, arginine and histidine; + for cellulose, casein, glycine, alanine, leucine, methionine and phenylalanine; and – for lignin, fructose, tryptophan and tyrosine.

Substrate	Concn (%)	
	0.2	0.02
Yeast extract + peptone	+++	++
Casamino acids	+	++
Maltose	++	+
Glucose	+	++
Galactose	+	++
Xylose	–	++
Mannitol	++	+
Inositol	++	+
Isoleucine	+	++
Valine	+	++
Serine	+	++
Threonine	+	++

7p75a^T culture grown in MJ synthetic seawater containing 0.2% (w/v) cellulose at 75 °C and pH 7.2 in the late exponential growth phase (data not shown). The finding of the extracellular cellulolytic activity suggested that the new isolate was capable of utilizing cellulose as the growth substrate. In addition, the utilization of many substrates depended upon concentration. High concentrations of casein, Casamino acids, cellobiose, glucose, galactose, xylose, isoleucine, valine, serine and threonine inhibited growth, while the growth rates and cell yields increased with increasing concentration with substrates such as maltose, mannitol, inositol, asparagine, glutamine, aspartate and glutamate (Table 1).

Fatty acid and DNA base compositions

The major cellular fatty acids of the new isolate grown at 75 °C were 52.3% iso-C_{17:0}, 27.6% C_{14:1}, 9.3% anteiso-C_{17:0}, 4.3% iso-C_{15:0}, 3.5% anteiso-C_{15:0}, 2.1% C_{16:0} and 0.9% C_{18:0}. The G + C content of the genomic DNA of strain 7p75a^T was 72.5 ± 0.5 mol%.

Phylogenetic analyses

The almost complete sequence (1488 bp) of the 16S rRNA gene from strain 7p75a^T was determined. The 16S rDNA sequence of the new isolate was repeatedly

Fig. 3. Phylogenetic tree of representative Gram-positive, low-G+C-content, spore-forming bacteria within the *Bacillus*–*Clostridium* subphylum inferred from 16S rDNA sequences by using the maximum-likelihood method of 1263 homologous positions of sequence from each organism. Numbers on tree represent the bootstrap value of the branching (1000 replicates). Numbers after the colons (:) are the G+C content in mol% of 1263 homologous positions of sequence. Scale bar indicates 5 substitutions per 100 nucleotides. The 16S rDNA sequences in this figure have GenBank accession numbers: *Thermotoga maritima* MSB8, M21774; *Clostridium thermocellum*, L09173; *Oxobacter pfennigii*, X77838; *Caloramator fervidus* RT4.B1, L09187; *Clostridium proteolyticum*, X73448; *Clostridium thermobutyricum* Wiegel, X72868; *Filifactor villosus*, X73452; *Clostridium xylanolyticum*, X76739; *Oxalophagus oxalicus*, Y14581; *Bacillus subtilis*, X60646; *Bacillus stearothermophilus* T10, X57309; *Desulfotomaculum australicum* AB33, M96665; *Desulfotomaculum thermobenzoicum*, L15628; *Anaerobranca horikoshii* JW/YL-138, U21809; *Thermosyntropha lipolytica* JW/VS-265, X99980; *Syntrophospora bryantii*, M26491; *Syntrophomonas wolfei*, AF022248; *Thermoanaerobacter marianensis* 7p75a^T, AB011495; *Moorella glycerini* JW/AS-Y6, U82327; *Moorella thermoacetica* LJD, M59121; *Moorella thermoautotrophicum* JW/701/3, L09168; *Dictyoglomus thermophilum* H-6-12, X69194; *Thermoanaerobacterium saccharolyticum* B6A-RI, L09169; *Thermoanaerobacterium thermosaccharolyticum* NCA 3814, M59119; *Thermoanaerobacter acetoethylicus* HTB2/W, L09164; *Thermoanaerobacter brockii* HTD4, L09165.

determined to check the culture purity and the reproducibility of the PCR-mediated rDNA sequence. Furthermore, the inspection of predicted secondary structures and evaluation by the CHECK-CHIMERA program of the RDP (Larsen *et al.*, 1993) indicated that the rDNA sequence of 7p75a^T was free of chimeric artifact (data not shown). Based on the sequence similarity analysis, the 16S rDNA sequence of the new isolate had less than 87% similarity with any other known sequences. The most similar rDNA sequences were those of *Moorella* (originally described as *Clostridium*) *thermoautotrophicum* (Wiegel *et al.*, 1981), *Moorella thermoacetica* (Fortaine *et al.*, 1942) and *Moorella glycerini* (Slobodkin *et al.*, 1997) with 85–87% similarity, and the rDNA sequences of the members of *Thermoanaerobacter* and *Desulfotomaculum* were also similar (80–85%). These findings implied that the new isolate was closely related to Gram-positive, low-G+C-content, spore-forming, anaerobic thermophilic bacteria within the *Bacillus*–*Clostridium* subphylum, even though the isolate appears to be phylogenetically distant from those

bacteria at the genus level. In order to assess the phylogenetic placement of the isolate, phylogenetic trees were reconstructed based on the neighbour-joining method and the maximum-likelihood method.

Phylogenetic analyses by the neighbour-joining and maximum-likelihood methods indicated identical topology in the affiliation of the new isolate, placing this organism close to the *Moorella* group but it represents a distinct branch showing early divergence from the *Moorella* group (Fig. 3). On the basis of its phylogenetic placement, the new isolate was the first obligate aerobe among obligate anaerobic bacteria. Although the estimated bootstrap confidence revealed low significance for the placement of 7p75a^T (Fig. 3), the base composition disparities in the 16S rDNA sequences among the Gram-positive, low-G+C-content, spore-forming, anaerobic thermophilic bacteria were relatively small (from 53.8 mol% G+C content in *Anaerobranca horikoshii* rDNA sequence to 62.0 mol% G+C content in the sequence of strain 7p75a^T) as compared to the base composition dis-

parities in the genomic DNAs (from 31.0 to 72.5 mol %) and had little influence on the tree topology, supported by transversion distance-matrix analysis (Woese *et al.*, 1991).

DISCUSSION

A strictly aerobic, heterotrophic, extremely thermophilic bacterium was isolated from the world's deepest sea-bottom, the Mariana Trench Challenger Deep at a depth of 10897 m. The results of 16S rDNA sequence analysis show that the new isolate is related to Gram-positive, low-G + C-content, spore-forming, anaerobic thermophilic bacteria including the members of the genera *Clostridium*, *Desulfotomaculum*, *Thermoanaerobacter*, *Thermoanaerobacterium*, *Anaerobranca*, *Thermosyntropha*, *Dictyoglomus* and *Moorella*, within the *Bacillus*–*Clostridium* subphylum (Wiegel & Ljungdahl, 1986; Lee *et al.*, 1993; Love *et al.*, 1993; Collins *et al.*, 1994; Engle *et al.*, 1995; Svetlitsnyi *et al.*, 1996; Slobodkin *et al.*, 1997; Stackebrandt *et al.*, 1997), but represents a distinct phylogenetic branch within this subphylum of bacteria (Fig. 3). The new isolate is the first aerobe growing strictly under aerobic conditions and is the most thermophilic bacterium, capable of growing at 80 °C, within these groups of thermophiles (Engle *et al.*, 1996; Liu *et al.*, 1996). Non-motile and lacking flagella, the cells are dissimilar to those of the most closely related *Moorella* species (Cato *et al.*, 1986; Slobodkin *et al.*, 1997), and spore-formation, a distinctive characteristic of anaerobic thermophilic bacteria within the *Bacillus*–*Clostridium* subphylum (Collins *et al.*, 1994), has not been observed with the new isolate. Furthermore, the high G + C content (72.5 mol %) of the genomic DNA of strain 7p75a^T is unusual among members of this group of Gram-positive bacteria with genomic DNA having a G + C content of 31–56 mol % (Cato *et al.*, 1986; Collins *et al.*, 1994; Engle *et al.*, 1996). The cellular fatty acid composition is as distinctive as compared to other Gram-positive bacteria (Cato *et al.*, 1986). On the basis of these physiological and molecular properties of the new isolate, we describe here a new genus, which is named *Thermaerobacter*. The type species is *Thermaerobacter marianensis* (named after the Mariana Trench), and the type strain is *Thermaerobacter marianensis* 7p75a^T (= JCM 10246^T).

Considering its requirement for sea salts for growth, *T. marianensis* is a typical marine bacterium. Other aerobic, heterotrophic, thermophilic bacteria which include *Thermus*, *Rhodothermus* and *Bacillus* species have been isolated and characterized from shallow and deep-sea hydrothermal systems (Kristjansson & Alfredsson, 1986; Alfredsson *et al.*, 1988; Sako *et al.*, 1996; Harmsen *et al.*, 1997). In addition, unnamed thermophilic bacteria different from these well-characterized genera have been isolated from deep-sea hydrothermal vents at the Mid-Atlantic Ridge and Guaymas Basin, and these include both Gram-positive and Gram-negative bacteria (Marteinsson *et al.*, 1995). Takami *et al.* (1997) have reported that unidentified

aerobic heterotrophic thermophiles exist in the surface mud of the Challenger Deep in the Mariana Trench. Hence, the discovery of this novel thermophilic bacterium *T. marianensis* supports the view that populations of novel and unidentified thermophilic bacteria may be widely distributed in deep-sea environments. In addition, considering sensitivity of *T. marianensis* to elevated hydrostatic pressure and the low temperature conditions of the Challenger Deep in the Mariana Trench, the new isolate is thought to have originated from shallow marine hydrothermal environments rather than from the deep-sea bottom. It has been shown that all of the thermophilic isolates from the Mariana Trench are strongly sensitive to elevated hydrostatic pressure as compared to mesophiles and psychrophiles (Takami *et al.*, 1997). Moreover, an extremely thermophilic strain growing at temperatures up to 80 °C, which has a 16S rDNA sequence with 98% similarity to that of *T. marianensis*, has been isolated from a shallow marine hydrothermal vent (Y. Sako, personal communication). It seems likely, therefore, that deep-sea environments are a depository of dormant thermophilic micro-organisms brought down from other hydrothermal habitats. Further exploration for deep-sea thermophilic bacteria will shed light on the undiscovered thermophiles and extend our understanding of microbial diversity in marine environments.

Description of *Thermaerobacter* gen. nov.

Thermaerobacter (Therm.ae.ro.bac'ter. Gr. adj. *thermos* hot; Gr. n. *aer* air; M.L. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod or staff; M.L. masc. n. *Thermaerobacter* rod which grows at high temperatures in the presence of air).

Rod-shaped, Gram-variable bacterium. Non-motile and no flagellum. Aerobic and thermophilic. Neutrophilic heterotroph. Requirement for sea salts for growth. Able to utilize various organic substrates such as yeast extract, peptone, cellulose, starch, chitin, casein, Casamino acids, a variety of sugars, carboxylic acids and amino acids. G + C content of genomic DNA is about 73 mol %. Major cellular fatty acids are iso-C_{17:0}, C_{14:1}, anteiso-C_{17:0}, iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0} and C_{18:0}. On the basis of 16S rRNA gene analysis, the genus *Thermaerobacter* is most closely related to the genus *Moorella* but does not belong to any of the taxa known so far. Members of the genus *Thermaerobacter* occur in not only marine hydrothermal vent environments but also the world's deepest sea-floor at a depth of 10897m. The type species is *Thermaerobacter marianensis*.

Description of *Thermaerobacter marianensis* sp. nov.

Thermaerobacter marianensis (ma.ri.a.nen'sis. M.L. masc. adj. *marianensis* pertaining to the Mariana Trench).

Straight to slightly curved rods, 0.3–0.6 × 2–7 µm, with rounded ends. Cells stain slightly Gram-positive in the

exponential growth phase but Gram-negative in the stationary growth phase. Occurs singly or in pairs. Exhibits no motility and no flagellum. Spores are not observed. Strictly aerobic. Temperature range for growth is 50–80 °C, with the optimum being 75 °C. pH range for growth is from 5.4 to 9.5, with optimum growth occurring at 7.0–7.5. Growth requires sea salts and occurs in the sea salts concentration range of 0.5–5.0% (w/v) with optimum growth occurring at 2.0%. Grows on yeast extract, peptone, starch, xylan, chitin, casein, Casamino acids, maltotriose, cellobiose, maltose, lactose, trehalose, saccharose, glucose, galactose, xylose, mannitol, inositol, propionate, 2-aminobutyric acid, malate, pyruvate, tartrate, succinate, lactate, acetate, glycerol, valine, isoleucine, cysteine, proline, serine, threonine, asparagine, glutamine, aspartate, glutamate, lysine, arginine or histidine and weakly on cellulose, glycine, alanine, leucine, methionine or phenylalanine. G+C content of genomic DNA is about 72.5 mol% (HPLC). Major cellular fatty acids are 52.3% iso-C_{17:0}, 27.6% C_{14:1}, 9.3% anteiso-C_{17:0}, 4.3% iso-C_{15:0}, 3.5% anteiso-C_{15:0}, 2.1% C_{16:0} and 0.9% C_{18:0}. 16S rDNA sequence exhibits 85–87% similarity to those of *Moorella thermoautotrophicum*, *Moorella thermoacetica* and *Moorella glycerini* and 80–85% similarity to rDNA sequences from the members of *Thermoanaerobacter* and *Desulfotomaculum*. Isolate was obtained from a mud sample from the bottom of the Challenger Deep in the Mariana Trench at a depth of 10 897 m. The type strain is *Thermaerobacter marianensis* 7p75a^T, = JCM 10246^T, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan.

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REFERENCES

- Alfredsson, G. A., Kristjansson, J. K., Hjorleifsdottir, S. & Stetter, K. O. (1988). *Rhodothermus marinus*, gen. nov., sp. nov., a thermophilic, halophilic bacterium from submarine hot springs in Iceland. *J Gen Microbiol* **134**, 49–68.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Amako, K., Murata, K. & Ueda, A. (1983). Structure of envelope of *Escherichia coli* observed by the rapid-freezing and substitution fixation method. *Microbiol Immunol* **27**, 95–99.
- Antoine, E., Cilia, V., Meunier, J. R., Guezennec, J., Lesongeur, F.

- & Barbier, G. (1997). *Thermosipho melanensis* sp. nov., a new thermophilic anaerobic bacterium belonging to the order *Thermotogales*, isolated from deep-sea hydrothermal vents in the southwestern Pacific Ocean. *Int J Syst Bacteriol* **47**, 1118–1123.
- Balch, W. E. & Wolfe, R. S. (1976). New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane-sulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl Environ Microbiol* **32**, 781–791.
- Baross, J. A. (1995). Isolation, growth, and maintenance of hyperthermophiles. In *Archaea: a Laboratory Manual. Thermophiles*, pp. 15–23. Edited by F. T. Robb & A. R. Place. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Blöchl, E., Rachel, R., Burggraf, S., Hafenbradl, D., Jannasch, H. W. & Stetter, K. O. (1997). *Pyrolobus fumarii*, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113 °C. *Extremophiles* **1**, 14–21.
- Brill, J. A. & Wiegel, J. (1997). Differentiation between spore-forming and asporogenic bacteria using a PCR and Southern hybridization based method. *J Microbiol Methods* **31**, 29–36.
- Brown, D. P., Genova-Raeva, L., Green, B. D., Wilkinson, S. R., Young, M. & Youngman, P. (1994). Characterization of *spo0A* homologs in diverse *Bacillus* and *Clostridium* species identifies DNA binding domain. *Mol Microbiol* **14**, 411–426.
- Canganella, F., Jones, W. J., Gambacorta, A. & Antranikian, G. (1997a). Biochemical and phylogenetic characterization of two novel deep-sea *Thermococcus* isolates with potentially biotechnological applications. *Arch Microbiol* **167**, 233–238.
- Canganella, F., Gonzalez, J. M., Yanagibayashi, M., Kato, C. & Horikoshi, K. (1997b). Pressure and temperature effects on growth and viability of the hyperthermophilic archaeon *Thermococcus peptonophilus*. *Arch Microbiol* **168**, 1–7.
- Cato, E. P., George, W. L. & Finegold, S. M. (1986). Genus *Clostridium*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1141–1200. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.
- DeLong, E. F. (1992). Archaea in coastal marine environments. *Proc Natl Acad Sci USA* **89**, 5685–5689.
- Engle, M., Li, Y., Woese, C. R. & Wiegel, J. (1995). Isolation and characterization of a novel alkalitolerant thermophile, *Anaerobranca horikoshii* gen. nov., sp. nov. *Int J Syst Bacteriol* **45**, 454–461.
- Engle, M., Li, Y., Rainey, F. A., DeBlois, S., Mai, V., Reichert, A., Mayer, F., Messner, P. & Wiegel, J. (1996). *Thermobrachium celere* gen. nov., sp. nov., a rapidly growing thermophilic, alkalitolerant, and proteolytic obligate anaerobe. *Int J Syst Bacteriol* **46**, 1025–1033.
- Fortaine, F. E., Peterson, W. H., McCoy, E., Johnson, M. J. & Ritter, G. J. (1942). A new type of glucose fermentation by *Clostridium thermoacetica* n. sp. *J Bacteriol* **43**, 701–715.
- Godfroy, A., Meunier, J.-R., Guezennec, J., Lesongeur, F., Raguene, G., Rimbault, A. & Barbier, G. (1996). *Thermococcus fumicolans* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent in the North Fiji Basin. *Int J Syst Bacteriol* **46**, 1113–1119.
- Godfroy, A., Lesongeur, F., Raguene, G., Querellou, J., Antoine,

- E., Meunier, J.-R., Guezennec, J. & Barbier, G. (1997). *Thermococcus hydrothermalis* sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **47**, 622–626.
- Gonzalez, J. M., Kato, C. & Horikoshi, K. (1995). *Thermococcus peptonophilus* sp. nov., a fast-growing, extremely thermophilic archaeobacterium isolated from deep-sea hydrothermal vents. *Arch Microbiol* **164**, 159–164.
- Harmsen, H. J. M., Prieur, D. & Jeanthon, C. (1997). Group-specific 16S rRNA-targeted oligonucleotide probes to identify thermophilic bacteria in marine hydrothermal vents. *Appl Environ Microbiol* **63**, 4061–4068.
- Huber, R., Wilharm, T., Huber, D., Trincone, A., Burggraf, S., Konig, H., Rachel, R., Rockinger, I., Fricke, H. & Stetter, K. O. (1992). *Aquifex pyrophilus* gen. nov. represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. *Syst Appl Microbiol* **15**, 340–351.
- Huber, H., Jannasch, H. W., Rachel, R., Fuchs, T. & Stetter, K. O. (1997). *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. *Syst Appl Microbiol* **20**, 374–380.
- Kato, C., Li, L., Tamaoka, J. & Horikoshi, K. (1997). Molecular analyses of the sediment of the 11000-m deep Mariana Trench. *Extremophiles* **1**, 117–123.
- Kawasumi, T., Igarashi, Y., Kodama, T. & Minoda, Y. (1984). *Hydrogenobacter thermophilus* gen. nov. and sp. nov., an extremely thermophilic, aerobic, hydrogen-oxidizing bacterium. *Int J Syst Bacteriol* **34**, 5–10.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* **16**, 111–120.
- Kristjansson, J. K. & Alfredsson, G. A. (1986). Life in Icelandic hot springs. *Naturufraedningurinn* **56**, 49–68.
- Larsen, N., Olsen, G. J., Maidak, B. L., McCaughey, M. J., Overbeek, R., Macke, T. J., Marsh, T. L. & Woese, C. R. (1993). The ribosomal database project. *Nucleic Acids Res* **21**, 3021–3023.
- Lauerer, G., Kristjansson, J. K., Langworthy, T. A., Konig, H. & Stetter, K. O. (1986). *Methanothermobacter sociabilis* sp. nov., a second species within the *Methanothermobacter* growing at 97 °C. *Syst Appl Microbiol* **8**, 100–105.
- Lee, Y., Jain, M. K., Lee, C., Lowe, S. E. & Zeikus, J. G. (1993). Taxonomic distinction of saccharolytic thermophilic anaerobes: description of *Thermoanaerobacterium xyloxyticum* gen. nov., sp. nov., and *Thermoanaerobacterium saccharolyticum* gen. nov., sp. nov.; reclassification of *Thermoanaerobium brockii*, *Clostridium thermosulfurigenes*, and *C. thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb. nov., *Thermoanaerobacterium thermosulfurigenes* comb. nov., and *Thermoanaerobacter thermohydrosulfuricum* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. *Int J Syst Bacteriol* **43**, 41–51.
- Liu, S.-Y., Rainey, F. A., Morgan, H. W., Mayer, F. & Wiegel, J. (1996). *Thermoanaerobacterium aotearoense* sp. nov., a slightly acidophilic, anaerobic thermophile isolated from various hot springs in New Zealand and emendation of the genus *Thermoanaerobacterium*. *Int J Syst Bacteriol* **46**, 388–396.
- Love, C. A., Patel, B. K. C., Stackebrandt, E. & Ludwig, W. (1993). The phylogenetic position of *Dictyoglomus* based on 16S rRNA sequence analysis. *FEMS Microbiol Lett* **107**, 317–320.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Marteinsson, V. T., Birrien, J.-L., Kristjansson, J. K. & Prieur, D. (1995). First isolation of thermophilic aerobic non-sporulating heterotrophic bacteria from deep-sea hydrothermal vents. *FEMS Microbiol Lett* **18**, 163–174.
- Mohr, S. C., Sokolov, N. V., He, C.-M. & Setlow, P. (1991). Binding of small, acid-soluble spore proteins from *Bacillus subtilis* changes the conformation of DNA from A to B. *Proc Natl Acad Sci USA* **88**, 77–81.
- Moriya, K., Inada, T., Kyo, M. & Horikoshi, K. (1995). Large-scale fermentation under high hydrostatic pressure using a newly developed deep-sea baro/thermophilic collection and cultivation system. *J Mar Biotechnol* **2**, 175–177.
- Olsen, G. J. (1988). Phylogenetic analysis using ribosomal RNA. *Methods Enzymol* **164**, 793–812.
- Padilla, J. A., Uno, F., Yamada, M., Namba, H. & Nii, S. (1997). High-resolution immuno-scanning electron microscopy using a non-coating method: study of herpes simplex virus glycoproteins on the surface of virus particles and infected cells. *J Electron Microsc* **46**, 171–180.
- Porter, K. G. & Feig, Y. S. (1980). The use of DAPI for identifying and counting microflora. *Limnol Oceanogr* **25**, 943–948.
- Sako, Y., Takai, K., Ishida, Y., Uchida, A. & Katayama, Y. (1996). *Rhodothermus obamensis* sp. nov., a modern lineage of extremely thermophilic marine bacteria. *Int J Syst Bacteriol* **46**, 1099–1104.
- Sanger, F., Coulson, A. R., Hong, G. F., Hill, O. F. & Petersen, G. B. (1982). Nucleotide sequence of bacteriophage λ DNA. *J Mol Biol* **162**, 729–773.
- Setlow, P. (1988). Small, acid-soluble spore proteins of *Bacillus* species: structure, synthesis, genetics function, and degradation. *Annu Rev Microbiol* **42**, 319–338.
- Slobodkin, A., Reysenbach, A.-L., Mayer, F. & Wiegel, J. (1997). Isolation and characterization of the homoacetogenic thermophilic bacterium *Moorella glycerini* sp. nov. *Int J Syst Bacteriol* **47**, 969–974.
- Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Pauker, O. & Hippe, H. (1997). Phylogenetic analysis of the genus *Desulfotomaculum*: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *Int J Syst Bacteriol* **47**, 1134–1139.
- Svetlitsnyi, V., Rainey, F. & Wiegel, J. (1996). *Thermosyntropho lipolyticum* gen. nov., sp. nov., a lipolytic, anaerobic, alkali-tolerant, thermophilic bacterium utilizing short- and long-chain fatty acids in syntrophic coculture with a methanogenic archaeum. *Int J Syst Bacteriol* **46**, 1131–1137.
- Takami, H., Inoue, A., Fuji, F. & Horikoshi, K. (1997). Microbial flora in the deepest sea mud of the Mariana Trench. *FEMS Microbiol Lett* **152**, 279–285.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Wiegel, J. & Ljungdahl, G. (1986). Genus *Thermoanaerobacter*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1141–1200. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Wiegel, J., Braun, M. & Gottschalk, G. (1981). *Clostridium thermoautotrophicum* sp. nov., a thermophile producing acetate from molecular hydrogen and carbon dioxide. *Curr Microbiol* **5**, 255–260.

Woese, C. R., Achenbach, L., Rouviere, P. & Mandelco, L. (1991). Archaeal phylogeny: re-examination of the phylogenetic position of *Archaeoglobus fulgidus* in light of certain composition induced artifacts. *Syst Appl Microbiol* **14**, 364–371.

Wood, P. J., Erfle, J. D. & Teather, R. M. (1988). Use of complex formation between Congo Red and polysaccharides in detection

and assay of polysaccharide hydrolases. *Methods in Enzymol* **160**, 59–74.

Zillig, W., Holz, I., Janekovic, D., Klenk, H.-P. & 7 other authors (1990). *Hyperthermus butylicus*, a hyperthermophilic sulfur-reducing archaeobacterium that ferments peptides. *J Bacteriol* **172**, 3959–3965.