Thermodesulfatator atlanticus sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a Mid-Atlantic Ridge hydrothermal vent.

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Electron micrographs of cells of strain AT1325^T (*Thermodesulfatator atlanticus* sp. nov.) (Fig S1) and a graph showing the effects of temperature on the maximum growth rate of the novel isolate (Fig. S2) are available in IJSEM Online.

A novel, strictly anaerobic, thermophilic and sulfate-reducing bacterium, strain AT1325^T, was isolated from a deep-sea hydrothermal vent at the Rainbow site on the Mid-Atlantic Ridge. This strain, designated AT1325^T, was subjected to a polyphasic taxonomic analysis. Its cells were Gram-negative motile rods (approximately 2.4 x 0.6 µm) with a single polar flagellum. Strain AT1325^T grew at temperatures between 55 and 75°C (optimum 65-70°C), from pH 5.5 to 8.0 (optimum 6.5-7.5) and between 1.5 and 4.5% (w/v) NaCl (optimum 2.5%). Cells grew chemolithoautotrophically with H_2 as

an energy source and SO_4^{2-} as an electron acceptor. Alternatively, the novel isolate was able to use methylamine, peptone or yeast extract as carbon sources. The dominant fatty acids (> 5%) detected in strain AT1325^T were $C_{16:0}$, $C_{18:1}\omega7c$, $C_{18:0}$ and $C_{19:0}$cyclo $\omega8c$. The G+C content of the genomic DNA was 45.6 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences placed strain AT1325^T within the family *Thermodesulfobacteriaceae*, in the bacterial domain. Comparative 16S rRNA gene sequence analysis indicated that strain AT1325^T belonged to the genus *Thermodesulfatator*, sharing 97.8% 16S rRNA sequence identity with *Thermodesulfatator indicus*, the unique representative species of this genus. On the basis of phylo-phenetic features, we propose a novel species, *Thermodesulfatator atlanticus* sp. nov. The type strain is AT1325^T (= DSM 21156^T = JCM 15391^T).

Over the past decades, microbiological investigations of a range of high-temperature marine and terrestrial environments have revealed the presence of a phylogenetically, metabolically and physiologically diverse community of thermophilic prokaryotes endemic to these particular habitats. Among the ubiquitous thermophilic taxa, members of the class *Thermodesulfobacteria* are commonly retrieved in hot biotopes, regardless the geographic provenance of the samples (Skirnisdottir *et al*., 2000; Nakagawa and Fukui, 2003; Nakagawa *et al*., 2005).

So far, the class *Thermodesulfobacteria* comprises only the family *Thermodesulfobacteriaceae*. Representatives of this family have been isolated from terrestrial geothermal hot springs, petroleum reservoirs and deep-sea hydrothermal vents located worldwide (Zeikus *et al*., 1983; Jeanthon *et al*., 2002; Kashefi *et al*., 2002; Moussard *et al*., 2004). Two recognized genera are currently described within the family *Thermodesulfobacteriaceae* (Hatchikian *et al*., 2002), namely the genus *Thermodesulfobacterium* (Zeikus *et al*., 1983) and the genus *Thermodesulfatator* (Moussard *et al*., 2004). These genera comprise exclusively thermophilic, anaerobic, chemoorganotrophic or chemolithoautotrophic sulfate-reducing strains. In addition, this family encompasses also the not-formally described species '*Geothermobacterium ferrireducens*' that is unable to reduce sulphate (Kashefi *et al*., 2002).

The genus *Thermodesulfatator* is so far composed of the unique species *T. indicus*, the type species being a strict chemolithoautotrophic sulfate-reducer that was isolated from a deep-sea hydrothermal vent from the Kairei vent field on the Central Indian Ridge (Moussard *et al*., 2004). In this study, a novel hydrothermal bacterium belonging to the genus *Thermodesulfatator* is described.

In June 2001, during the ATOS oceanographic cruise, fragments of active hydrothermal chimney rocks were collected from 2275m depth at the Rainbow vent field, on the Mid-Atlantic Ridge (36°13'N, 33°54'W). Sample collection, subsampling and storage procedures were as described elsewhere (Postec *et al*., 2005). All subsamples were pooled and used to inoculate a 2L gas-lift bioreactor (inoculum 2% v/v). A continuous enrichment culture was performed in this bioreactor for 41 days, at 60°C and pH 6.5, on a complex medium (modified SME medium) containing sea water salts (including sulfate), minerals, carbohydrates, peptides, organic acids (acetate and pyruvate), and colloidal sulfur (Postec *et al*., 2007). This continuous culture was performed, at a dilution rate of 0.04 h^{-1} (80 mL h^{-1}), and under a stream of N_2 (0.1 v v^{-1} min^{-1}) to maintain anaerobic conditions and to drain possible inhibitors. Then, a culture sample from day 28 was used as an initial inoculum on TYA medium (60°C, pH 6.0, sulfate 9.3 mM, atmosphere of H_2/CO_2 80/20 2 bars), as described by Postec *et al*. (2007). One strain was purified by both isolation on solid medium (TYA medium solidified with 1.5 % Phytagel incubated in anaerobic jars under H_2/CO_2 80/20 2 bars) and then repeated dilutions-to-extinction series. This strain referenced as strain AT1325^T is described in this publication.
Purity of this isolate was confirmed routinely by microscopic examination and by repeated partial sequencing of the 16S rRNA gene using several primers. Stock cultures were stored at −80°C in TYA medium supplemented with 5% (v/v) DMSO.

The 16S rRNA gene (1491 bp) of the novel isolate was double-strand sequenced as described elsewhere (Alain *et al*., 2002). This sequence was first compared to those in available databases by use of the BLAST program (Altschul *et al*., 1990). It was then aligned to its nearest neighbours using the CLUSTALX program (Thompson *et al*., 1997). The alignment was refined manually using the SEAVIEW program (Galtier *et al*., 1996) based on an alignment generated in parallel with the RDP II sequence aligner (http://rdp8.cme.msu.edu/cgis/seq_align.cgi). 1395 nucleotides corresponding to homologous regions could

be unambiguously aligned by the RDP II Sequence Align program and were used for subsequent calculations of identity percentages (similarity matrix). Afterwards, phylogenetic reconstructions were calculated by the PHYLIP (PHYLogeny Inference Package) version 3.67 software (http://evolution.genetics.washington.edu/phylip/getme.html) on the basis of evolutionary distance (neighbour-joining method – NJ- with Jukes and Cantor corrections) (Saitou and Nei, 1987) and maximum likelihood ML (Felsenstein, 1981). The robustness of the inferred topologies was assessed by bootstrap analyses (1000 bootstrap resamplings with NJ and 100 replications with ML) (Felsenstein, 1985). Comparison of the 16S rRNA gene sequence of strain AT1325^T with sequences of *Bacteria* revealed a phylogenetic relationship with deeply branching bacterial lineages. Phylogenetic reconstructions indicated that the novel isolate belonged unquestionably to the class *Thermodesulfobacteria* (Garrity and Holt, 2001) and more especially to the family *Thermodesulfobacteriaceae,* order *Thermodesulfobacteriales* (Hatchikian *et al*., 2002). Within this lineage, the novel isolate clustered robustly with *T. indicus* (Moussard *et* al., 2004), an other deep-sea hydrothermal vent isolate (Fig. 1). Both isolates shared 97.8% 16S rRNA gene sequence identity. Strain AT1325^T was most distantly related to members of the genera *Thermodesulfobacterium* and '*Geothermobacterium',* sharing 87.7 to 88.7% 16S rRNA gene sequence identity with representatives of these genera. Based on the sequence identity and phylogenetic analyses, the novel isolate could be assigned to the genus *Thermodesulfatator.* The level of 16S rRNA gene sequence dissimilarity with *Thermodesulfatator indicus* also showed that the novel isolate displayed sufficient molecular differences for a delineation at the species-level (Stackebrandt and Ebers, 2006). Indeed, the sequence similarity between the 16S rRNA genes of *T. indicus* and strain AT1325 is far below the threshold value (98.7-99%) currently recommended to perform DNA-DNA hybridization in order to test for the genomic uniqueness of a novel isolate (Stackebrandt and Ebers, 2006).

Morphological characteristics of cells of strain AT1325^T were determined by light microscopy (Olympus CX40), transmission electron microscopy (Jeol JEM 100 CX II) and scanning electron microscopy (FEI Quanta 200). Cells were straight rods of 1.0-6.1 µm in length (mean 2.4 µm ± 1.5, n=10) and 0.3-0.8 µm in width (mean 0.6 ± 0.1, n=10) in the mid-exponential phase of growth (see supplementary Fig. S1A and S1B in IJSEM Online). They stained Gram-negative. Cells occurred mainly singly and divided by constriction

(Fig. S1A). They possessed single polar flagellum (Fig. S1B) and were highly motile. Formation of spores was never observed.

The physiological characterization of the novel isolate was carried out in a basal medium referenced as "SO4PNsalts" and containing (per liter): 0.33 g NH_4Cl, 0.5 g KCl, 0.5 g $CaCl_2.2H_2O$, 3 g $MgCl_2.6H_2O$, 22 g NaCl, 3.0 g Na_2SO_4, 5 g PIPES buffer (Sigma) and 1 mg resazurin (Sigma). Its pH was adjusted to 6.7. Once prepared, this medium was autoclaved and cooled to room temperature under a O_2-free N_2 gas flow. Then, 1 ml vitamin mixture [solution from Widdel & Bak (1992) supplemented with 4 mg folic acid and 1.5 mg lipoic acid], 1 ml thiamine solution (Widdel & Bak,1992), 1 ml selenite-tungstate solution (Widdel & bak, 1992) and 1 ml non-chelated trace element mixture (Widdel & Bak, 1992) were added to the basal medium from concentrated anaerobic filter-sterilized solutions. Finally, KH_2PO_4 was injected from sterile stock to a final concentration of 40 mM. Medium (10 ml) was then dispensed anaerobically in 50 ml vials sealed with butyl-rubber stoppers and reduced with 0.1 ml of a 10% (w/v) $Na_2S.9H_2O$ sterile solution, just before inoculation. Unless stated otherwise, the experiments were carried out anaerobically, under a gas phase of H_2/CO_2 (80/20; 200 kPa), and incubation were done in the dark, at 65°C.

Growth was routinely monitored by direct cell counting using a modified Thoma chamber (depth 10 μm), or by counting after fixation with 1% (v/v) glutaraldehyde and storage at –20°C. Growth rates were calculated using linear regression analysis of five to nine points along the linear portions of the growth curves logarithmically-transformed. The determination of the temperature range for growth was tested over the range 50-85°C (i.e. 50, 55, 60, 65, 70, 75, 80 and 85°C). No growth was observed at 50°C, 80°C and above. The novel isolate grew from 55 to 75°C, with an optimum growth rate at 65-70°C (see Supplementary Fig. S2 in IJSEM Online). The pH range for growth was tested from initial pH 4.0 to initial pH 9.0, at 65°C, in basal medium buffered and adjusted to the required pH (initial pH at 20 °C) as described elsewhere (Alain *et al*., 2002). Growth was observed from pH 5.5 to pH 8.0, the optimum being around pH 6.5-7.5. No growth was observed at pH 5.0 and below, neither at pH 8.5 and above. Salt tolerance was tested at 65°C in 'SO4PNsalts' medium prepared with various concentrations of NaCl (0, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0 and 10% w/v). Growth was observed at salt concentrations ranging from 1.5 to 4.5% (w/v)

NaCl, the optimum salinity being around 2.5%. No growth was observed at 1 and 5 % (w/v) NaCl. Under optimal growth conditions, the generation time of strain AT1325^T was around 3 hours and 20 minutes.

The determination of the whole-cell fatty acid composition was performed on cultures grown at 70°C on "SO4PNsalts" medium, under a gas phase of H_2/CO_2 (80/20; 200 kPa). The production of biomass was done both in media prepared with and without 0.1 g L^{-1} yeast extract. In both cases, cells were harvested at the end of the exponential phase of growth. In parallel, *Thermodesulfatator indicus* str. CIR29812^T was grown exactly under the same conditions and in the same culture medium in order to compare its PFLA pattern. The analyses were carried out at the DSMZ according to the standard protocol of the Microbial Identification System (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996). Results are detailed in Table 1. Similarly to *T. indicus*, the fatty acid profile of strain AT1325^T comprised hydroxylated fatty acids, cyclic fatty acids, saturated and unsaturated straight chain fatty acids, that consisted mainly of $C_{16:0}$, $C_{18:0}$ and $C_{18:1}\omega7c$. However, the fatty acids profiles of the novel isolate and of *T. indicus* str. CIR29812^T grown exactly under the same conditions displayed few minor differences as shown in Table 1.

Strain AT1325^T was a strict anaerobic, chemolithoautotrophic bacterium that used hydrogen and sulfate as respective primary electron donor and acceptor. Its ability to use alternative electron acceptors was tested by adding elemental sulfur (12 g l^{-1}), L-cystine (12 g l^{-1}), sulfite (1 mM), thiosulfate (20 mM), nitrate (10 mM), nitrite (1 mM) or oxygen (1% v/v) to sulfate-depleted media, under a H_2/CO_2 atmosphere (80/20; 200 kPa). Quantitative determination of hydrogen sulphide was determined as described elsewhere (Cord-Ruwisch, 1985). The novel isolate was found to reduce sulfate to H_2S, but did not grow when sulfur, L-cystine, sulfite, thiosulfate, nitrate, nitrite and oxygen were used as electron acceptors. For a sulfate-reducer, the inability to use sulfite as sole terminal electron acceptor is surprising but is not an exception within the microbial world (Itoh *et al.*, 1999; Jeanthon *et al.*, 2002; Moussard *et al.*, 2004). To examine possible carbon sources other than CO_2, a variety of organic carbon sources were tested in the presence of sulfate, under an atmosphere of H_2 100% (200 kPa). Formate (10 mM), acetate (20 mM), propionate (20 mM), methanol (0.5% v/v), pyruvate (10 mM), glucose (20 mM), monomethylamine (10 mM), peptone (0.2 g l^{-1}) and yeast extract (0.2

g l^{-1}) were tested as potential substrates. Significant growth coupled to hydrogen sulphide production was still observed after three transfers on the same medium (inoculation to 1/200 in all cases) when monomethylamine, peptone or yeast extract were provided as sole carbon source and when H_2 and sulfate were the respective electron donor and acceptor. Under the conditions tested, formate, acetate, propionate, methanol, pyruvate and glucose could not be used as sole carbon source. In order to compare the physiological capabilities of the novel isolate to the ones of its closest relative *Thermodesulfatator indicus* str. CIR29812^T, with respect to carbon source utilization, carbon source tests were performed in parallel with *T. indicus* under exactly the same conditions. Under our experimental conditions, *T. indicus* str. CIR29812^T did not grow when monomethylamine, peptone or yeast extract were provided as sole carbon source and when H_2 and sulfate were the respective electron donor and acceptor. To test for the capability of the strain AT1325^T to use electron donors other than molecular hydrogen, the strain was cultivated under a gas phase of N_2/CO_2 (80/20%, 200 kPa) in the presence of formate (10 mM), acetate (20 mM), butyrate (10 mM), lactate (10 mM), methanol 0.5% (v/v), yeast extract (0.2 g l^{-1}) and peptone (0.2 g l^{-1}) with sulfate as a terminal electron acceptor. No growth was observed with the alternative energy sources, indicating that strain AT1325^T was a strict hydrogen-oxidizer. Finally, the nitrogen sources for growth were also examined in a nitrogen-depleted medium. The novel isolate was found to grow on organic and inorganic nitrogen sources. Significant growth was observed when NH_4Cl (20 mM), glutamate (10 mM), yeast extract (0.2 g l^{-1}), tryptone (0.2 g l^{-1}), gelatin (0.05% v/v) and urea (0.05% v/v) were provided as sole nitrogen source.

Antibiotic resistance was tested in the presence of a variety of antibiotics from different chemical nature and with different targets and mechanisms. The resistance to vancomycin, streptomycin, chloramphenicol, kanamycin, rifampicin, penicillin G, ampicillin and tetracycline was investigated at concentrations of 10, 50, 100 and 200 μg ml^{-1}. When the antibiotic was diluted in ethanol (chloramphenicol) or DMSO (rifampicin), the same volume of solvent was added to control cultures rather than water. All antibiotics were added aseptically before inoculation and the cultures were incubated at 65°C for one week. Strain AT1325^T was found to be sensitive to 10 μg ml^{-1} of ampicillin and penicillin G. It grew in the presence of 10 μg ml^{-1} vancomycin and tetracycline, of 50 μg ml^{-1} rifampicin and chloramphenicol, but was sensitive to higher

concentrations of these four antibiotics. The novel isolate was resistant to 200 μg ml^{-1} streptomycin and kanamycin.

The G+C content of the genomic DNA was determined from the melting point according to Marmur & Doty (1962), as described elsewhere (Alain *et al*., 2002). A calibration curve was constructed by use of ultrapure DNA from *Clostridium perfringens* (26.5 mol% G+C), calf thymus (42 mol% G+C), *Escherichia coli* strain B (50 mol% G+C) and *Micrococcus luteus* (72 mol% G+C) as standards (Sigma). The G+C content of strain AT1325^T was 45.6 mol%.

In summary, the novel isolate shares many physiological, chemotaxonomic and metabolic properties with its closest relative *T. indicus.* Its phenotypic and genotypic properties generally met the characteristics described for the genus *Thermodesulfatator* (Moussard *et al*., 2004). Indeed, strain AT1325^T is a marine, thermophilic, strictly anaerobic bacterium growing chemolithoautrophically from H_2 oxidation and using sulfate as sole electron acceptor. It robustly branches with the unique representative of the genus *Thermodesulfatator*, namely *T. indicus*. Nevertheless, strain AT1325^T can be distinguished from *T. indicus* and from other *Thermodesulfobacteriaceae* species in terms of a number of genotypic and physiological features detailed in Table 2. In brief, the novel taxon differs from *T. indicus* by its clear phylogenetic distance and its broader pH range for growth. In addition, the novel isolate is able to use some organic compounds (methylamine, peptone and yeast extract) as sole carbon source while its congener *T. indicus* is unable. In conclusion, in view of the above-mentioned distinctive features, we propose that the isolate AT1325^T should be assigned as the type strain of a novel species, for which the name *Thermodesulfatator atlanticus* sp. nov. is proposed.

Description of *Thermodesulfatator atlanticus* sp. nov.

Thermodesulfatator atlanticus (at.lan'ti.cus. L. masc. adj. *atlanticus*, from the Atlantic Ocean, referring to the site of isolation of the type strain).

Cells are Gram-negative motile rods (1.04–6.08 μm long by 0.30–0.75 μm wide) with a single polar flagellum. Optimal growth occurs at 65-70°C, with a growth range from 55 to 75°C. The pH and NaCl ranges are 5.5-8.0 (optimum 6.5-7.5) and 1.5-4.5% (w/v) (optimum, 2.5% w/v NaCl), respectively. Growth occurs under strictly anaerobic conditions using

H_2 as an electron donor, sulfate as a terminal electron acceptor and CO_2 as a carbon source. Strict hydrogen-oxidizer. The following are not used as electron donors: formate, acetate, lactate, methanol, peptone and yeast extract. In the presence of H_2 and sulfate, monomethylamine, peptone or yeast extract can be used as sole carbon source. No growth is observed when formate, acetate, propionate, methanol, pyruvate and glucose are provided as sole carbon source. Does not ferment pyruvate or lactate. The following are not utilized as electron acceptors: elemental sulfur, L-cystine, thiosulfate, sulfite, nitrate, nitrite, oxygen. Is able to utilize a wide range of organic and inorganic nitrogen sources. Antibiotic resistance: resistant to 200 μg ml^{-1} streptomycin and kanamycin; Sensitive to 10 μg ml^{-1} ampicillin and penicillin G; sensitive to 50 μg ml^{-1} vancomycin and tetracycline, and to 100 μg ml^{-1} rifampicin and chloramphenicol. Fatty acid profile is mainly composed of $C_{16:0}$, $C_{18:1}\omega 7c$, $C_{18:0}$ and $C_{19:0}$cyclo $\omega 8c$. Genomic DNA G+C content of the type strain $AT1325^T$ is 45.6 mol%.

The type strain, $AT1325^T$ ($DSM\ 21156^T$, $JCM\ 15391^T$) was isolated from the walls of an active deep-sea hydrothermal vent chimney at the Rainbow vent field, on the Mid-Atlantic Ridge (36°13'N, 33°54'W). It is also available under request at the "Souchothèque de Bretagne" (catalogue LMBE) culture collection (http://www.ifremer.fr/souchotheque/).

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TABLES and FIGURES

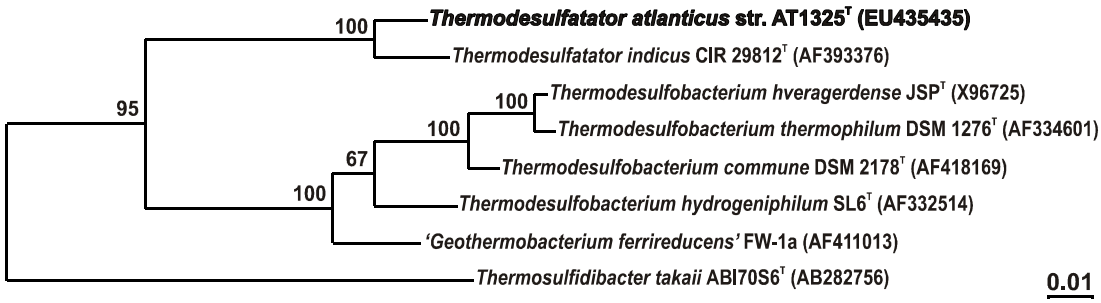

Fig. 1. **Phylogenetic relationships of strain AT1325^T and its closest relatives within the class *Thermodesulfobacteria***. 16S rRNA gene sequence data of reference strains were obtained from the GenBank/EMBL databases. Accession numbers are indicated in brackets. The topology shown corresponds to an unrooted tree obtained by the Maximum Likelihood algorithm, established using the PHYLIP package. Bootstrap values (from 100 replicates) are indicated at the branch nodes. The positioning of the novel isolate was confirmed by the Neighbour-Joining method. The scale bar indicates 1 nt substitutions per 100 nt.

Table 1. Whole cell fatty acid profiles of strain AT1325^T and of *Thermodesulfatator indicus* strain CIR29812^T.

Values are percentages of total fatty acids. The nomenclature is as follows: the first number indicates the number of carbon atoms in the molecule. The prefixes 'anteiso', 'iso', 'OH' and 'cyclo' indicate anteiso- or iso-branched, hydroxy or cyclic fatty acids. The second number following the colon indicates the number of double bonds. The position of the double bond is indicated by the carbon atom position starting from the methyl (ω) end of the molecule. The suffix *c* indicates the *cis* isomer. Summed feature contain one or more of each fatty acid. Summed features: **3**, $C_{16:1}\omega7c$ and/or 2-OH iso-$C_{15:0}$; **5**, $C_{18:2}\omega6,9c$ and/or anteiso $C_{18:0}$. Major fatty acids (>5%) are indicated in bold values. ND: not detected.

Fatty acid	**Strain AT1325^T grown on "SO4PNsalts" medium (under a gas phase of H_2/CO_2 and without yeast extract)**
Saturated fatty acids	
$C_{16:0}$	**6.37**
$C_{17:0}$	1.12
$C_{18:0}$	**16.14**
Monounsaturated fatty acids	
$C_{16:1}\omega5c$	0.82
$C_{17:1}\omega6c$	2.23
$C_{18:1}\omega9c$	1.16
$C_{18:1}\omega7c$	**59.43**
$C_{18:1}\omega5c$	2.07
$C_{20:1}\omega7c$	1.23
Hydroxyl fatty acid	
3-OH $C_{16:0}$	1.90
Cyclic fatty acid	
$C_{19:0}$ cyclo $\omega8c$	**6.40**

Summed features	
Summed feature 3	1.13

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Fatty acid	**Strain AT1325^T**	***Thermodesulfatator indicus* str. CIR29812^T**
	Both strains were grown exactly under the same conditions on "SO4PNsalts" medium, in the presence of 0.1 g/L yeast extract and under a H_2/CO_2 atmosphere	
Saturated fatty acids		
$C_{12:0}$	0.56	0.40
$C_{14:0}$	1.26	0.76
$C_{15:0}$	ND	0.59
$C_{16:0}$ N alcohol	ND	0.19
iso $C_{16:0}$	0.49	0.39
$C_{16:0}$	**21.79**	**19.61**
anteiso $C_{17:0}$	0.22	ND
$C_{17:0}$	2.27	**10.04**
iso $C_{18:0}$	0.24	ND
$C_{18:0}$	**33.87**	**29.82**
$C_{19:0}$	0.27	1.16
$C_{20:0}$	0.81	0.35
Monounsaturated fatty acids		
$C_{16:1}\omega5c$	0.24	0.20
$C_{17:1}\omega6c$	1.88	3.62
$C_{17:1}\omega8c$	0.19	0.35
$C_{18:1}$ iso H	0.39	0.31
$C_{18:1}\omega9c$	4.08	2.68
$C_{18:1}\omega7c$	**21.76**	**21.63**
$C_{18:1}\omega5c$	0.77	0.71
$C_{18:3}\omega6c$ (6, 9, 12)	0.30	0.28
$C_{20:1}\omega7c$	> max ar/ht	ND
Hydroxyl fatty acid		
3-OH $C_{16:0}$	1.08	0.42
3-OH $C_{18:0}$	0.38	ND
Cyclic fatty acid		
$C_{19:0}$ cyclo $\omega8c$	4.42	4.34
Summed features		
Summed feature 3	1.29	1.16
Summed feature 5	1.46	0.97

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337 **Table 2. Characteristics differentiating strain AT1325^T from representative species of the family**
338 ***Thermodesulfobacteriaceae*.** Species: 1, *Thermodesulfatator atlanticus* AT1325^T (this study); 2,
339 *Thermodesulfatator indicus* (Moussard *et al*., 2004); 3, *Thermodesulfobacterium hydrogeniphilum*
340 (Jeanthon *et al*., 2002); 4, *Thermodesulfobacterium commune* (Zeikus *et al*., 1983); 5, '*Geothermobacterium*
341 *ferrireducens*' (Kashefi *et al*., 2002).

342 Legend: +, positive; ─, negative; ND, not determined. The percentage of 16S rRNA gene sequence identity
343 is calculated in reference to the 16S rRNA gene sequence of the novel isolate AT1325^T.

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Characteristic	1	2	3	4	5
Temperature range for growth (°C) [optimum]	55-75 [65-70]	55-80 [70]	50-80 [75]	45-82 [70]	65-100 [85-90]
pH range for growth [optimum]	5.5-8.0 [6.5-7.5]	6.0-6.7 [6.25]	6.3-6.8 [6.5]	6.0-8.0 [7.0]	ND [6.8-7.0]
NaCl concentration range for growth (%) [optimum]	1.5-4.5 [2.5]	1.0-3.5 [2.5]	0.5-5.5 [3.0]	0-2.0 [0]	0-0.75 [0-0.05]
Carbon sources					
CO_2	+	+	+	–	+
Organic compounds	+	–	–	+	–
Electron donors					
H_2	+	+	+	–	+
Pyruvate	–	–	–	+	–
Lactate	–	–	–	+	–
Electron acceptors					
Sulfate	+	+	+	+	–
Thiosulfate	–	–	–	+	–
Iron (III)	ND	ND	ND	ND	+
Fermentation	–	–	–	+	–
DNA G+C content (mol%)	45.6	46	28	34.4	ND
16S rRNA gene sequence identity (%)	100	97.8	88.7	87.7	88.7

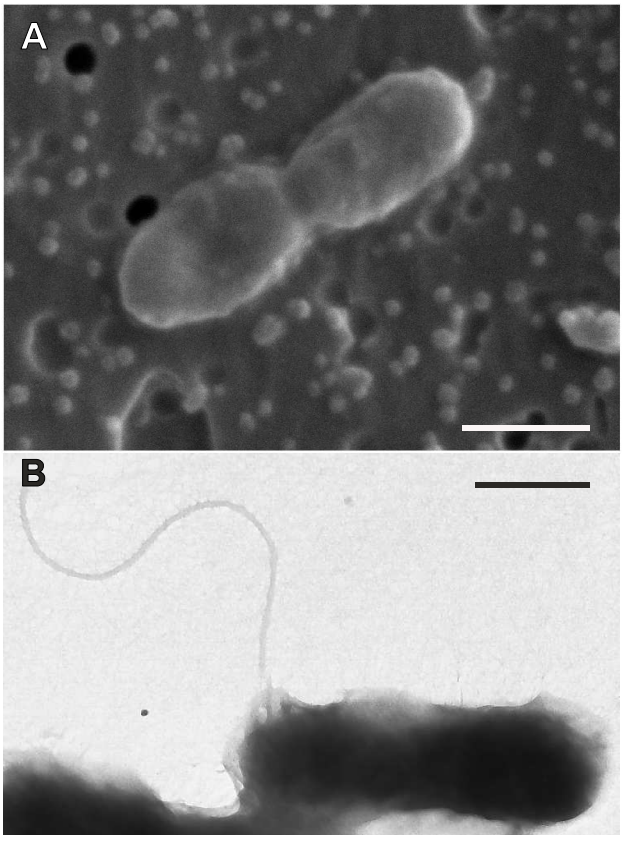

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Fig. S1. Scanning and transmission electron micrographs of cells of strain AT1325^{T} in the mid-exponential phase of growth, showing the division by constriction (A) and the polar flagellum (B). Bars, 1.0 and 0.5μm.

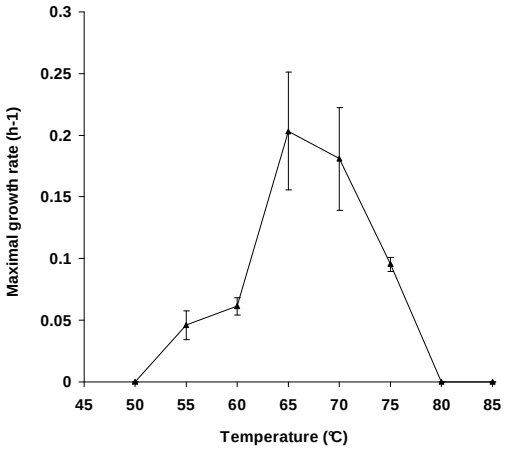

Fig. S2. Effects of temperature on the maximum growth rate of strain AT1325^{T}. Bars indicate confidence intervals.