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1 *Thermodesulfatator atlanticus* sp. nov.,

2 **a novel thermophilic chemolithoautotrophic sulfate-reducing bacterium isolated from a Mid-Atlantic**
3 **Ridge hydrothermal vent**

4
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13
14 Running title: *Thermodesulfatator atlanticus* sp. nov.

15
16 Category: Other Bacteria

17
18 Footnote: The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of
19 *Thermodesulfatator atlanticus* AT1325^T is EU435435.

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

23
24 **A novel, strictly anaerobic, thermophilic and sulfate-reducing bacterium, strain AT1325^T, was**
25 **isolated from a deep-sea hydrothermal vent at the Rainbow site on the Mid-Atlantic Ridge. This**
26 **strain, designated AT1325^T, was subjected to a polyphasic taxonomic analysis. Its cells were Gram-**
27 **negative motile rods (approximately 2.4 x 0.6 µm) with a single polar flagellum. Strain AT1325^T grew**
28 **at temperatures between 55 and 75°C (optimum 65-70°C), from pH 5.5 to 8.0 (optimum 6.5-7.5) and**
29 **between 1.5 and 4.5% (w/v) NaCl (optimum 2.5%). Cells grew chemolithoautotrophically with H₂ as**

30 an energy source and SO_4^{2-} as an electron acceptor. Alternatively, the novel isolate was able to use
31 methylamine, peptone or yeast extract as carbon sources. The dominant fatty acids (> 5%) detected in
32 strain AT1325^T were C_{16:0}, C_{18:1} ω 7c, C_{18:0} and C_{19:0}cyclo ω 8c. The G+C content of the genomic DNA
33 was 45.6 mol%.

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

40
41 Over the past decades, microbiological investigations of a range of high-temperature marine and terrestrial
42 environments have revealed the presence of a phylogenetically, metabolically and physiologically diverse
43 community of thermophilic prokaryotes endemic to these particular habitats. Among the ubiquitous
44 thermophilic taxa, members of the class *Thermodesulfobacteria* are commonly retrieved in hot biotopes,
45 regardless the geographic provenance of the samples (Skirnisdottir *et al.*, 2000; Nakagawa and Fukui, 2003;
46 Nakagawa *et al.*, 2005).

47
48 So far, the class *Thermodesulfobacteria* comprises only the family *Thermodesulfobacteriaceae*.
49 Representatives of this family have been isolated from terrestrial geothermal hot springs, petroleum
50 reservoirs and deep-sea hydrothermal vents located worldwide (Zeikus *et al.*, 1983; Jeanthon *et al.*, 2002;
51 Kashefi *et al.*, 2002; Moussard *et al.*, 2004). Two recognized genera are currently described within the
52 family *Thermodesulfobacteriaceae* (Hatchikian *et al.*, 2002), namely the genus *Thermodesulfobacterium*
53 (Zeikus *et al.*, 1983) and the genus *Thermodesulfatator* (Moussard *et al.*, 2004). These genera comprise
54 exclusively thermophilic, anaerobic, chemoorganotrophic or chemolithoautotrophic sulfate-reducing strains.
55 In addition, this family encompasses also the not-formally described species '*Geothermobacterium*
56 *ferrireducens*' that is unable to reduce sulphate (Kashefi *et al.*, 2002).

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

61

62 In June 2001, during the ATOS oceanographic cruise, fragments of active hydrothermal chimney rocks were
63 collected from 2275m depth at the Rainbow vent field, on the Mid-Atlantic Ridge (36°13'N, 33°54'W).
64 Sample collection, subsampling and storage procedures were as described elsewhere (Postec *et al.*, 2005).
65 All subsamples were pooled and used to inoculate a 2L gas-lift bioreactor (inoculum 2% v/v). A continuous
66 enrichment culture was performed in this bioreactor for 41 days, at 60°C and pH 6.5, on a complex medium
67 (modified SME medium) containing sea water salts (including sulfate), minerals, carbohydrates, peptides,
68 organic acids (acetate and pyruvate), and colloidal sulfur (Postec *et al.*, 2007). This continuous culture was
69 performed, at a dilution rate of 0.04 h⁻¹ (80 mL h⁻¹), and under a stream of N₂ (0.1 v v⁻¹ min⁻¹) to maintain
70 anaerobic conditions and to drain possible inhibitors. Then, a culture sample from day 28 was used as an
71 initial inoculum on TYA medium (60°C, pH 6.0, sulfate 9.3 mM, atmosphere of H₂/CO₂ 80/20 2 bars), as
72 described by Postec *et al.* (2007). One strain was purified by both isolation on solid medium (TYA medium
73 solidified with 1.5 % Phytigel incubated in anaerobic jars under H₂/CO₂ 80/20 2 bars) and then repeated
74 dilutions-to-extinction series. This strain referenced as strain AT1325^T is described in this publication.

75 Purity of this isolate was confirmed routinely by microscopic examination and by repeated partial
76 sequencing of the 16S rRNA gene using several primers. Stock cultures were stored at -80°C in TYA
77 medium supplemented with 5% (v/v) DMSO.

78

79 The 16S rRNA gene (1491 bp) of the novel isolate was double-strand sequenced as described elsewhere
80 (Alain *et al.*, 2002). This sequence was first compared to those in available databases by use of the BLAST
81 program (Altschul *et al.*, 1990). It was then aligned to its nearest neighbours using the CLUSTALX program
82 (Thompson *et al.*, 1997). The alignment was refined manually using the SEAVIEW program (Galtier *et al.*,
83 1996) based on an alignment generated in parallel with the RDP II sequence aligner
84 (http://rdp8.cme.msu.edu/cgis/seq_align.cgi). 1395 nucleotides corresponding to homologous regions could

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

107

108 Morphological characteristics of cells of strain AT1325^T were determined by light microscopy (Olympus
109 CX40), transmission electron microscopy (Jeol JEM 100 CX II) and scanning electron microscopy (FEI
110 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
111 width (mean 0.6 ± 0.1, n=10) in the mid-exponential phase of growth (see supplementary Fig. S1A and S1B
112 in IJSEM Online). They stained Gram-negative. Cells occurred mainly singly and divided by constriction

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

115

116 The physiological characterization of the novel isolate was carried out in a basal medium referenced as
117 “SO4PNsalts” and containing (per liter): 0.33 g NH₄Cl, 0.5 g KCl, 0.5 g CaCl₂·2H₂O, 3 g MgCl₂·6H₂O, 22 g
118 NaCl, 3.0 g Na₂SO₄, 5 g PIPES buffer (Sigma) and 1 mg resazurin (Sigma). Its pH was adjusted to 6.7. Once
119 prepared, this medium was autoclaved and cooled to room temperature under a O₂-free N₂ gas flow. Then, 1
120 ml vitamin mixture [solution from Widdel & Bak (1992) supplemented with 4 mg folic acid and 1.5 mg
121 lipoic acid], 1 ml thiamine solution (Widdel & Bak,1992), 1 ml selenite-tungstate solution (Widdel & bak,
122 1992) and 1 ml non-chelated trace element mixture (Widdel & Bak, 1992) were added to the basal medium
123 from concentrated anaerobic filter-sterilized solutions. Finally, KH₂PO₄ was injected from sterile stock to a
124 final concentration of 40 mM. Medium (10 ml) was then dispensed anaerobically in 50 ml vials sealed with
125 butyl-rubber stoppers and reduced with 0.1 ml of a 10% (w/v) Na₂S·9H₂O sterile solution, just before
126 inoculation. Unless stated otherwise, the experiments were carried out anaerobically, under a gas phase of
127 H₂/CO₂ (80/20; 200 kPa), and incubation were done in the dark, at 65°C.

128

129 Growth was routinely monitored by direct cell counting using a modified Thoma chamber (depth 10 μm), or
130 by counting after fixation with 1% (v/v) glutaraldehyde and storage at -20°C. Growth rates were calculated
131 using linear regression analysis of five to nine points along the linear portions of the growth curves
132 logarithmically-transformed. The determination of the temperature range for growth was tested over the
133 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.
134 The novel isolate grew from 55 to 75°C, with an optimum growth rate at 65-70°C (see Supplementary Fig.
135 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
136 basal medium buffered and adjusted to the required pH (initial pH at 20 °C) as described elsewhere (Alain *et*
137 *al.*, 2002). Growth was observed from pH 5.5 to pH 8.0, the optimum being around pH 6.5-7.5. No growth
138 was observed at pH 5.0 and below, neither at pH 8.5 and above. Salt tolerance was tested at 65°C in
139 ‘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,
140 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)

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

143 The determination of the whole-cell fatty acid composition was performed on cultures grown at 70°C on
144 “SO4PNsalts” medium, under a gas phase of H₂/CO₂ (80/20; 200 kPa). The production of biomass was done
145 both in media prepared with and without 0.1 g L⁻¹ yeast extract. In both cases, cells were harvested at the
146 end of the exponential phase of growth. In parallel, *Thermodesulfatator indicus* str. CIR29812^T was grown
147 exactly under the same conditions and in the same culture medium in order to compare its PFLA pattern.
148 The analyses were carried out at the DSMZ according to the standard protocol of the Microbial
149 Identification System (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett Packard model
150 HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer &
151 Kroppenstedt (1996). Results are detailed in Table 1. Similarly to *T. indicus*, the fatty acid profile of strain
152 AT1325^T comprised hydroxylated fatty acids, cyclic fatty acids, saturated and unsaturated straight chain
153 fatty acids, that consisted mainly of C_{16:0}, C_{18:0} and C_{18:1ω7c}. However, the fatty acids profiles of the novel
154 isolate and of *T. indicus* str. CIR29812^T grown exactly under the same conditions displayed few minor
155 differences as shown in Table 1.

156

157 Strain AT1325^T was a strict anaerobic, chemolithoautotrophic bacterium that used hydrogen and sulfate as
158 respective primary electron donor and acceptor. Its ability to use alternative electron acceptors was tested by
159 adding elemental sulfur (12 g l⁻¹), L-cystine (12 g l⁻¹), sulfite (1 mM), thiosulfate (20 mM), nitrate (10 mM),
160 nitrite (1 mM) or oxygen (1% v/v) to sulfate-depleted media, under a H₂/CO₂ atmosphere (80/20; 200 kPa).
161 Quantitative determination of hydrogen sulphide was determined as described elsewhere (Cord-Ruwisch,
162 1985). The novel isolate was found to reduce sulfate to H₂S, but did not grow when sulfur, L-cystine, sulfite,
163 thiosulfate, nitrate, nitrite and oxygen were used as electron acceptors. For a sulfate-reducer, the inability to
164 use sulfite as sole terminal electron acceptor is surprising but is not an exception within the microbial world
165 (Itoh *et al.*, 1999; Jeanthon *et al.*, 2002; Moussard *et al.*, 2004). To examine possible carbon sources other
166 than CO₂, a variety of organic carbon sources were tested in the presence of sulfate, under an atmosphere of
167 H₂ 100% (200 kPa). Formate (10 mM), acetate (20 mM), propionate (20 mM), methanol (0.5% v/v),
168 pyruvate (10 mM), glucose (20 mM), monomethylamine (10 mM), peptone (0.2 g l⁻¹) and yeast extract (0.2

169 g l⁻¹) were tested as potential substrates. Significant growth coupled to hydrogen sulphide production was
170 still observed after three transfers on the same medium (inoculation to 1/200 in all cases) when
171 monomethylamine, peptone or yeast extract were provided as sole carbon source and when H₂ and sulfate
172 were the respective electron donor and acceptor. Under the conditions tested, formate, acetate, propionate,
173 methanol, pyruvate and glucose could not be used as sole carbon source. In order to compare the
174 physiological capabilities of the novel isolate to the ones of its closest relative *Thermodesulfatator indicus*
175 str. CIR29812^T, with respect to carbon source utilization, carbon source tests were performed in parallel with
176 *T. indicus* under exactly the same conditions. Under our experimental conditions, *T. indicus* str. CIR29812^T
177 did not grow when monomethylamine, peptone or yeast extract were provided as sole carbon source and when
178 H₂ and sulfate were the respective electron donor and acceptor. To test for the capability of the strain
179 AT1325^T to use electron donors other than molecular hydrogen, the strain was cultivated under a gas phase
180 of N₂/CO₂ (80/20%, 200 kPa) in the presence of formate (10 mM), acetate (20 mM), butyrate (10 mM),
181 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
182 terminal electron acceptor. No growth was observed with the alternative energy sources, indicating that
183 strain AT1325^T was a strict hydrogen-oxidizer. Finally, the nitrogen sources for growth were also examined
184 in a nitrogen-depleted medium. The novel isolate was found to grow on organic and inorganic nitrogen
185 sources. Significant growth was observed when NH₄Cl (20 mM), glutamate (10 mM), yeast extract (0.2 g l⁻¹)
186 ¹), tryptone (0.2 g l⁻¹), gelatin (0.05% v/v) and urea (0.05% v/v) were provided as sole nitrogen source.

187

188 Antibiotic resistance was tested in the presence of a variety of antibiotics from different chemical nature and
189 with different targets and mechanisms. The resistance to vancomycin, streptomycin, chloramphenicol,
190 kanamycin, rifampicin, penicillin G, ampicillin and tetracycline was investigated at concentrations of 10, 50,
191 100 and 200 µg ml⁻¹. When the antibiotic was diluted in ethanol (chloramphenicol) or DMSO (rifampicin),
192 the same volume of solvent was added to control cultures rather than water. All antibiotics were added
193 aseptically before inoculation and the cultures were incubated at 65°C for one week. Strain AT1325^T was
194 found to be sensitive to 10 µg ml⁻¹ of ampicillin and penicillin G. It grew in the presence of 10 µg ml⁻¹
195 vancomycin and tetracycline, of 50 µg ml⁻¹ rifampicin and chloramphenicol, but was sensitive to higher

196 concentrations of these four antibiotics. The novel isolate was resistant to 200 µg ml⁻¹ streptomycin and
197 kanamycin.

198

199 The G+C content of the genomic DNA was determined from the melting point according to Marmur & Doty
200 (1962), as described elsewhere (Alain *et al.*, 2002). A calibration curve was constructed by use of ultrapure
201 DNA from *Clostridium perfringens* (26.5 mol% G+C), calf thymus (42 mol% G+C), *Escherichia coli* strain
202 B (50 mol% G+C) and *Micrococcus luteus* (72 mol% G+C) as standards (Sigma). The G+C content of strain
203 AT1325^T was 45.6 mol%.

204

205 In summary, the novel isolate shares many physiological, chemotaxonomic and metabolic properties with its
206 closest relative *T. indicus*. Its phenotypic and genotypic properties generally met the characteristics
207 described for the genus *Thermodesulfatator* (Moussard *et al.*, 2004). Indeed, strain AT1325^T is a marine,
208 thermophilic, strictly anaerobic bacterium growing chemolithoautotrophically from H₂ oxidation and using
209 sulfate as sole electron acceptor. It robustly branches with the unique representative of the genus
210 *Thermodesulfatator*, namely *T. indicus*. Nevertheless, strain AT1325^T can be distinguished from *T. indicus*
211 and from other *Thermodesulfobacteriaceae* species in terms of a number of genotypic and physiological
212 features detailed in Table 2. In brief, the novel taxon differs from *T. indicus* by its clear phylogenetic
213 distance and its broader pH range for growth. In addition, the novel isolate is able to use some organic
214 compounds (methylamine, peptone and yeast extract) as sole carbon source while its congener *T. indicus* is
215 unable. In conclusion, in view of the above-mentioned distinctive features, we propose that the isolate
216 AT1325^T should be assigned as the type strain of a novel species, for which the name *Thermodesulfatator*
217 *atlanticus* sp. nov. is proposed.

218

219 **Description of *Thermodesulfatator atlanticus* sp. nov.**

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

222 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
223 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)
224 and 1.5-4.5% (w/v) (optimum, 2.5% w/v NaCl), respectively. Growth occurs under strictly anaerobic conditions using

225 H₂ as an electron donor, sulfate as a terminal electron acceptor and CO₂ as a carbon source. Strict hydrogen-oxidizer.
226 The following are not used as electron donors: formate, acetate, lactate, methanol, peptone and yeast extract. In the
227 presence of H₂ and sulfate, monomethylamine, peptone or yeast extract can be used as sole carbon source. No growth is
228 observed when formate, acetate, propionate, methanol, pyruvate and glucose are provided as sole carbon source. Does
229 not ferment pyruvate or lactate. The following are not utilized as electron acceptors: elemental sulfur, L-cystine,
230 thiosulfate, sulfite, nitrate, nitrite, oxygen. Is able to utilize a wide range of organic and inorganic nitrogen sources.
231 Antibiotic resistance: resistant to 200 µg ml⁻¹ streptomycin and kanamycin; Sensitive to 10 µg ml⁻¹ ampicillin and
232 penicillin G; sensitive to 50 µg ml⁻¹ vancomycin and tetracycline, and to 100 µg ml⁻¹ rifampicin and chloramphenicol.
233 Fatty acid profile is mainly composed of C_{16:0}, C_{18:1}*ω*7*c*, C_{18:0} and C_{19:0}*cyclo ω*8*c*. Genomic DNA G+C content of the
234 type strain AT1325^T is 45.6 mol%.

235

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

239

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248 **REFERENCES**

- 249 **Alain, K., Querellou, J., Lesongeur, F., Pignet, P., Crassous, P., Raguénès, G., Cueff, V. & Cambon-Bonavita,**
250 **M.-A. (2002).** *Caminibacter hydrogeniphilus* gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium
251 isolated from an East Pacific Rise hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1317-1323.
- 252 **Altschul, S., Gish, W., Miller, W., Myers, E. & Lipman, D. (1990).** Basic local alignment search tool. *J Mol Biol*
253 **215**, 403-410.
- 254 **Cord-Ruwisch, R. (1985).** A quick method for the determination of dissolved and precipitated sulfides in cultures of
255 sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33-36.
- 256 **Felsenstein, J. (1981).** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368-
257 376.
- 258 **Felsenstein, J. (1985).** Confidence limits on phylogenies: an approach using the bootstrap. *Evol* **30**, 783-791.
- 259 **Galtier, N., Gouy, M. & Gautier, C. (1996).** SEAVIEW and PHYLO_WIN: two graphic tools for sequence alignment
260 and molecular phylogeny. *CABIOS* **12**, 543-548.
- 261 **Garrity, G. M. & Holt, J. G. (2001).** Phylum BIII. *Thermodesulfobacteria* phy. nov. In *Bergey's Manual of Systematic*
262 *Bacteriology*, 2nd edn, vol. 1, pp. 389-393. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York:
263 Springer.
- 264 **Hatchikian, E. C., Ollivier, B. & Garcia, J. J. (2002).** Family I. *Thermodesulfobacteriaceae* fam. nov. In *Bergey's*
265 *Manual of Systematic Bacteriology*, 2nd edn, vol. 1, p. 390. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity.
266 New York: Springer.
- 267 **Itoh, T., Suzuki, K.-I., Sanchez, P. C., Nakase, T. (1999).** *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus
268 of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *Int J Syst Bacteriol* **49**, 1157-1163.
- 269 **Jeanthon, C., L'Haridon, S., Cueff, V., Banta, A., Reysenbach, A.-L. & Prieur, D. (2002).** *Thermodesulfobacterium*
270 *hydrogeniphilum* sp. nov., a thermophilic, chemolithoautotrophic, sulfate-reducing bacterium isolated from a deep-sea
271 hydrothermal vent at Guaymas Basin, and emendation of the genus *Thermodesulfobacterium*. *Int J Syst Evol Microbiol*
272 **52**, 765-772.
- 273 **Kämpfer, P. & Kroppenstedt, R. M. (1996).** Numerical analysis of fatty acid patterns of coryneform bacteria and
274 related taxa. *Can J Microbiol* **42**, 989-1005.
- 275 **Kashefi, K., Holmes, D. E., Reysenbach, A.-L. & Lovley, D. R. (2002).** Use of Fe(III) as an electron acceptor to
276 recover previously uncultured hyperthermophiles: isolation and characterization of *Geothermobacterium ferrireducens*
277 gen. nov. *Appl Environ Microbiol* **68**, 1735-1742.

278 **Marmur, J. & Doty, P. (1962).** Determination of the base composition of desoxyribonucleic acid from its thermal
279 denaturation temperature. *J Mol Biol* **5**, 109-118.

280 **Moussard, H., L'Haridon, S., Tindall, B.J., Banta, A., Schumann, P., Stackebrandt, E., Reysenbach, A.-L. &**
281 **Jeanthon, C. (2004).** *Thermodesulfatator indicus* gen. nov., sp. nov., a novel thermophilic chemolithoautotrophic
282 sulfate-reducing bacterium isolated from the Central Indian Ridge. *Int J Syst Evol Microbiol* **54**, 227-233.

283 **Nakagawa, T. & Fukui, M. (2003).** Molecular characterization of community structures and sulfur metabolism within
284 microbial streamers in Japanese hot springs. *Appl Environ Microbiol* **69**, 7044-7057.

285 **Nakagawa, S., Takai, K., Inagaki, F., Chiba, H., Ishibashi, J., Kataoka, S., Hirayama, H., Nunoura, T.,**
286 **Horikoshi, K. & Sako, Y. (2005).** Variability in microbial community and venting chemistry in a sediment-hosted
287 backarc hydrothermal system: impacts of seafloor phase-separation. *FEMS Microbiol Ecol* **54**, 141-155.

288 **Postec, A., Urios, L., Lesongeur, F., Ollivier, B., Querellou, J. & Godfroy, A. (2005).** Continuous enrichment culture
289 and molecular monitoring to investigate the microbial diversity of thermophiles inhabiting deep-sea hydrothermal
290 ecosystems. *Current Microbiol* **50**, 1-7.

291 **Postec, A., Lesongeur, F., Pignet, P., Ollivier, B., Querellou, J. & Godfroy, A. (2007).** Continuous enrichment
292 cultures : insights into prokaryotic diversity and metabolic interactions in deep-sea vent chimneys. *Extremophiles* **11**,
293 747-757.

294 **Saitou, N. & Nei, M. (1987).** The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol*
295 *Biol Evol* **4**, 406-425.

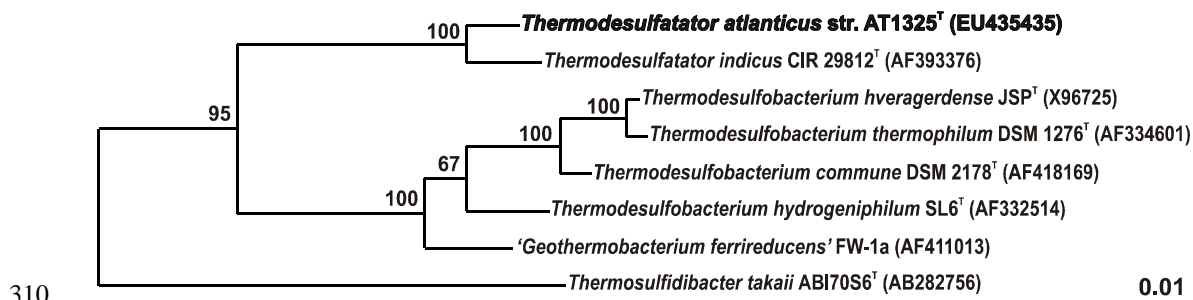
296 **Skirnisdottir, S., Hreggvidsson, G. O., Hjörleifsdottir, S., Marteinson, V. T., Petursdottir, S. K., Holst, O. &**
297 **Kristjansson, J. K. (2000).** Influence of sulfide and temperature on species composition and community structure of
298 hot spring microbial mats. *Appl Environ Microbiol* **66**, 2835-2841.

299 **Stackebrandt, E. & Ebers, J. (2006).** Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**,
300 152-155.

301 **Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. (1997).** The ClustalX windows
302 interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**,
303 4876-4882.

304 **Widdel, F. & Bak, F. (1992).** Gram-negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes*. Balows, A.,
305 Trüper, H.G., Dworkin, M., Harder, W. and Schleifer, K.-H. (eds). New York, USA: Springer, pp. 3352-3378.

306 **Zeikus, J. G., Dawson, M. -A., Thompson, T. E., Ingvorsen, K. & Hatchikian, E. C. (1983).** Microbial ecology of
307 volcanic sulphidogenesis: isolation and characterization of *Thermodesulfobacterium commune* gen. nov. and sp. nov. *J*
308 *Gen Microbiol* **129**, 1159-1169.



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

318

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

321 Values are percentages of total fatty acids. The nomenclature is as follows: the first number indicates the
 322 number of carbon atoms in the molecule. The prefixes ‘anteiso’, ‘iso’, ‘OH’ and ‘cyclo’ indicate anteiso- or
 323 iso-branched, hydroxy or cyclic fatty acids. The second number following the colon indicates the number of
 324 double bonds. The position of the double bond is indicated by the carbon atom position starting from the
 325 methyl (ω) end of the molecule. The suffix *c* indicates the *cis* isomer. Summed feature contain one or more
 326 of each fatty acid. Summed features: **3**, C_{16:1} ω 7*c* and/or 2-OH iso-C_{15:0}; **5**, C_{18:2} ω 6,9*c* and/or anteiso C_{18:0}.
 327 Major fatty acids (>5%) are indicated in bold values. ND: not detected.

328

Fatty acid	Strain AT1325 ^T grown on “SO ₄ PNsalts” 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} ω 5 <i>c</i>	0.82
C _{17:1} ω 6 <i>c</i>	2.23
C _{18:1} ω 9 <i>c</i>	1.16
C_{18:1}ω7<i>c</i>	59.43
C _{18:1} ω 5 <i>c</i>	2.07
C _{20:1} ω 7 <i>c</i>	1.23
Hydroxyl fatty acid	
3-OH C _{16:0}	1.90
Cyclic fatty acid	
C _{19:0} cyclo ω 8 <i>c</i>	6.40

Summed features	
Summed feature 3	1.13

329

Fatty acid	Strain AT1325 ^T	<i>Thermodesulfatator indicus</i> 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} ω5 <i>c</i>	0.24	0.20
C _{17:1} ω6 <i>c</i>	1.88	3.62
C _{17:1} ω8 <i>c</i>	0.19	0.35
C _{18:1} iso H	0.39	0.31
C _{18:1} ω9 <i>c</i>	4.08	2.68
C_{18:1}ω7<i>c</i>	21.76	21.63
C _{18:1} ω5 <i>c</i>	0.77	0.71
C _{18:3} ω6 <i>c</i> (6, 9, 12)	0.30	0.28
C _{20:1} ω7 <i>c</i>	> 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 ω8 <i>c</i>	4.42	4.34
Summed features		
Summed feature 3	1.29	1.16
Summed feature 5	1.46	0.97

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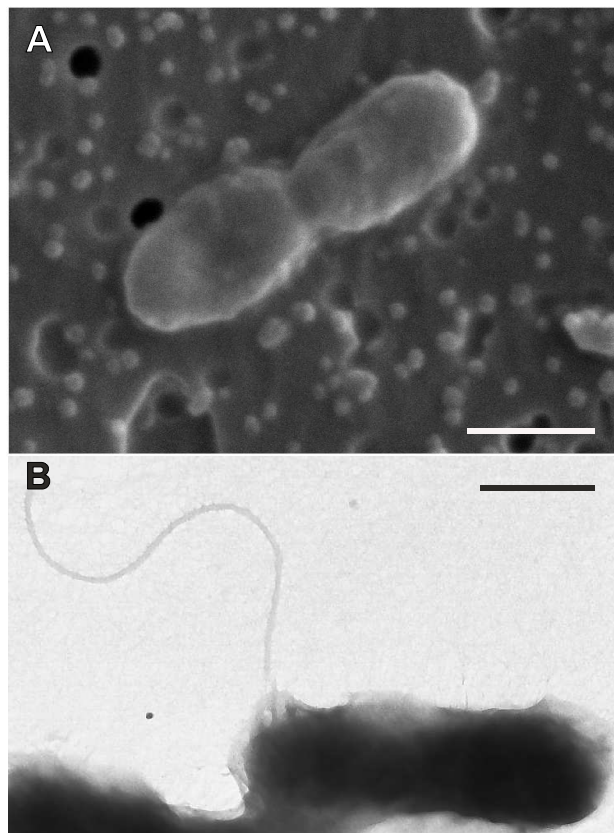
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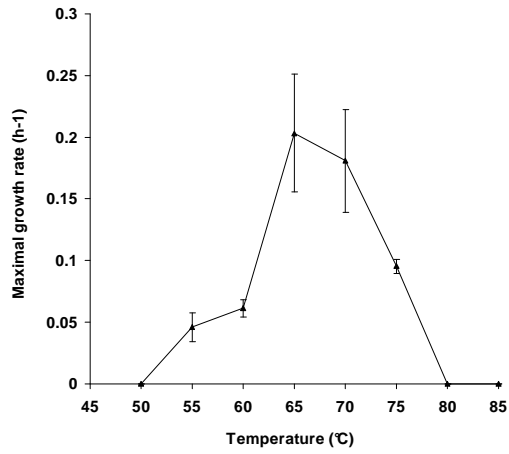
337 **Table 2. Characteristics differentiating strain AT1325^T from representative species of the family**
338 ***Thermodesulfobacteriaceae*.** Species: 1, *Thermodesulfatator atlanticus* AT1325^T (this study); 2,
339 *Thermodesulfatator indicus* (Moussard *et al.*, 2004); 3, *Thermodesulfobacterium 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.

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



346 **Fig. S1. Scanning and transmission electron micrographs of cells of strain AT1325^T** in the mid-
347 exponential phase of growth, showing the division by constriction (A) and the polar flagellum (B). Bars, 1.0
348 and 0.5 μm .
349



350
351 **Fig. S2. Effects of temperature on the maximum growth rate of strain AT1325^T.** Bars indicate
352 confidence intervals.