

1 ***Thermococcus thioeducens* sp. nov., a novel hyperthermophilic, obligately**
2 **sulfur-reducing archaeon from a deep-sea hydrothermal vent**

3
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24 A hyperthermophilic, sulfur-reducing, organo-heterotrophic archaeon, strain
25 OGL-20P^T, was isolated from “black smoker” chimney material from the
26 Rainbow hydrothermal vent site on the Mid-Atlantic Ridge (36.2 °N, 33.9 °W).
27 The cells of strain OGL-20P^T have an irregular coccoid shape and are motile
28 with a single flagellum. Growth was observed within the pH range 5.0–8.5
29 (optimum pH 7.0), NaCl concentration range 1-5 % (w/v) (optimum 3 %), and
30 temperature range 55-94 °C (optimum 83-85 °C). The novel isolate is strictly
31 anaerobic and obligately dependent upon elemental sulfur as an electron
32 acceptor, but it does not reduce sulfate, sulfite, thiosulfate, iron (III) or nitrate.
33 Proteolysis products (peptone, bacto-tryptone, casamino-acids, and yeast
34 extract) are utilized as substrates during sulfur-reduction. Strain OGL-20P^T is
35 resistant to ampicillin, chloramphenicol, kanamycin, and gentamycin, but
36 sensitive to tetracycline and rifampicin. The G+C content of DNA is 52.9 mol%.
37 The 16S rRNA gene sequence analysis revealed that strain OGL-20P^T is closely
38 related to *Thermococcus coalescens* and related species, but no significant
39 homology by DNA-DNA hybridization was observed between those species and
40 the new isolate. On the basis of physiological and molecular properties of the
41 new isolate, we conclude that strain OGL-20P^T represents a new separate species
42 within the genus *Thermococcus*, and propose the name *Thermococcus*
43 *thioreducens* sp. nov. The type strain is OGL-20P^T (= ATCC BAA-394^T = JCM
44 12859^T = DSM 14981^T).

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46 The Gen Bank accession number for the 16S rRNA gene sequence of strain

47 OGL-20P^T is AF 394925.

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49 The genus *Thermococcus* was created in 1983, and currently 25 species have been
50 validly published. All members of this genus are characterized by a thermophilic
51 nature, anaerobiosis with sulfur-type respiration and sometimes sulfur stimulation for
52 fermentation (Zillig, 1992; Zillig & Reysenbach, 2002). The typical ecological systems
53 for the habitat of *Thermococcus* species include geothermal springs (volcanic
54 fumaroles, geysers, and deep-sea hydrothermal vents), deep subsurface biosphere such
55 as deep crustal rocks and aquifers and high-temperature oil wells (Stetter *et al.*, 1993;
56 Takahata *et al.*, 2000; Miroshnichenko *et al.*, 2001). Most species of the genus
57 *Thermococcus* are marine and have an optimum NaCl concentration of about 3 %
58 (w/v), but there are also fresh-water species, e.g. *T. zilligii* (Ronimus *et al.*, 1997) and
59 *T. waiotapuensis* (González *et al.*, 1999). Most members of the *Thermococcus* genus
60 grow optimally at neutral or slightly acidic pH, and only *T. alkaliphilus* is capable of
61 growth at pH 10.5 with optimum around 9.0 (Keller *et al.*, 1995). The minimum
62 temperature for growth of *Thermococcus* is 50 °C and the maximum is about 95 °C as
63 for *T. celer*, *T. litoralis*, and *T. fumicolans* (Zillig *et al.*, 1983; Neuner *et al.*, 1990;
64 Godfroy *et al.*, 1996). Many species of the genus *Thermococcus* have been isolated
65 from deep-sea hydrothermal vents with environmental pressures in excess of 200
66 atmospheres. Obligate dependence upon pressure was determined at 95-100 °C for *T.*
67 *barophilus* (Marteinsson *et al.*, 1999). The most radioresistant hyperthermophilic
68 archaeon, *T. gammatolerans*, is capable of surviving 30 kGy γ -ray irradiation (Jolivet
69 *et al.*, 2003). Most species of the genus *Thermococcus* are sulfur reducing organisms,

70 however, Slobodkin *et al.* (1999) reported dissimilatory reduction of Fe(III) by
71 *Thermococcus* sp.T642. In this article we describe a novel hyperthermophilic archaeon
72 *Thermococcus thioeducens* sp. nov., which is an obligate sulfur-reducer, and was
73 isolated from the Rainbow deep-sea hydrothermal vent site in the Mid-Atlantic Ridge.
74
75 “Black Smoker” chimney material samples were collected in October 1999 from
76 2,300 meter depth in the Rainbow hydrothermal vent field (36.2 °N; 33.9 °W) about
77 800 km southwest of the Azores on the Azorean segment of the Mid-Atlantic Ridge.
78 Remote manipulators (on the *Mir* submersible launched from the Russian
79 oceanographic research vessel *Akademik Mstislav Keldysh*) were used to place the
80 samples on a collection tray for return to the surface. After a brief exposure to the
81 ambient atmosphere during the submersible recovery out of the water, the samples
82 were hermetically sealed in sterile vessels with screw caps and maintained at 4 °C in
83 an insulated cooler during transport to the Astrobiology Laboratory of the NASA,
84 Marshall Space Flight Center. Strain OGL-20P^T was isolated from a sample of black
85 colored fine-grained sand and mud (neutral pH, 3 % (w/v) salinity) that contained
86 chimney debris material and organic sediments.
87 The enrichment, isolation, and cultivation of the new isolate were performed in a
88 liquid medium under a highly purified 100 % nitrogen atmosphere. The basal medium
89 contained g l⁻¹: KH₂PO₄, 0.3; MgCl₂·6H₂O, 0.1; KCl, 0.3; NH₄Cl, 1.0; NaHCO₃, 0.2;
90 CaSO₄·7H₂O, 0.005; NaCl, 30.0; Na₂S·9H₂O, 0.4; yeast extract, 0.5; sulfur powder,
91 10.0, peptone, 5.0, and resazurin, 0.001. The medium was supplemented with 2 ml of
92 vitamin solution (Wolin *et al.*, 1963) and 1 ml of trace element solution as described

93 earlier (Pikuta *et al.*, 2000). The final pH^{22C} of the medium after autoclaving was 7.2-
94 7.4.

95 Unless otherwise noted, enrichment and pure cultures were grown in 10 ml of medium
96 in Hungate tubes under one atmosphere of N₂ (100 %). All transfers and samplings of
97 cultures were performed with sterile syringes. The medium was sterilized at 121 °C for
98 60 min and after adding sulfur to the tubes under an atmosphere of 100 % nitrogen an
99 additional sterilization was performed at 110 °C for 30 min. All incubations for
100 physiology description were carried out at 83 °C. One half gram of sample L-20 was
101 injected into the medium and incubated for 24 h. A pure culture of strain OGL-20P^T
102 was obtained after repeated serial dilutions. The culture on the 10⁻⁹ dilution with the
103 monotypic morphology was chosen for the following “roll-tube” serial dilutions
104 purification. Growth of colonies occurred after 2-3 days incubation on 3 % (w/v) Difco
105 agar in Hungate tubes at 70 °C. One colony on the 10⁻⁸ dilution tube was chosen for
106 consequent purification and designated as strain OGL-20P^T. The colonies of strain
107 OGL-20P^T on the surface of the agar were whitish-cream in color, glossy and shining,
108 with a round shape (~1.5 mm diameter), irregular cleaved edges and convex with
109 denser raised conic center. In deep agar, colonies had a convex-convex lenticular
110 shape.

111 Phase-contrast microscopy revealed the cells of strain OGL-20P^T were irregular,
112 motile cocci with diameter 0.7 to 1.7 μm. Some of the time the cells looked as
113 diplococci or conglomerates of 10-15 cells. Transmission Electron Microscopy was
114 carried out using a JEOL TEM 100 CX II operating at 80 kV. Negative staining was

115 performed using a uranyl acetate procedure as described previously (Pikuta *et al.*,
116 2003). TEM images showed the presence of a single flagellum (Fig. 1).
117 Culture growth was measured by direct cell counting under a phase-contrast
118 microscope (Fisher Micromaster, USA), by measuring sulfide produced from sulfur in
119 the process of growth (Truper & Schlegel, 1964), or by estimating an increase in
120 optical density at 595 nm (Genesis 5; Spectronic Instruments, USA). The pH of the
121 medium was adjusted to defined values with sterile stock solutions of 6 N HCl or 6 N
122 NaOH under a flow of N₂ and measured using a pH meter (model 230 Aplus, Orion,
123 USA) calibrated at 22 °C. All measurements were performed after cooling the culture
124 samples to room temperature. The temperature range for growth was determined in the
125 liquid medium at pH 7.3. The effect of NaCl concentration on growth was determined
126 in the liquid medium containing 0.0, 0.5, 1.0, 2.0, 3.0, 5.0, 7.0, and 10.0 % (w/v) NaCl.
127 NaCl requirement was studied using a modified medium, in which NaHCO₃ was
128 replaced with K₂CO₃ and Na₂S was replaced with K₂S. Growth of strain OGL-20P^T
129 was observed in the temperature range of 55 to 95 °C, with optimum between 83 and
130 85 °C. Strain OGL-20P^T survived during 30 minutes at 101 °C, but incubation at 103
131 °C during 2 h killed the cells. Growth of strain OGL-20P^T was observed within the pH
132 range of 5.0-8.5, with optimum pH at 7.0; within NaCl concentration range of 1 to 5 %
133 (w/v) with optimum of 3 % (w/v). No growth was detected for NaCl concentrations
134 below 0.5 % or above 7 % (w/v). The doubling time measured by direct cell counting
135 under a phase-contrast microscope for a fresh culture of OGL-20P^T incubated at
136 optimal conditions was 30 minutes.

137 Strain OGL-20P^T was found to be strictly anaerobic. The catalase activity, which was
138 tested as described by Smilbert & Krieg (1994), showed negative reaction. The
139 utilization of various electron acceptors was studied in a medium containing peptone
140 (5g l⁻¹) as an electron donor. Electron acceptors were added in the form of autoclaved
141 or filter-sterilized stock solutions. The final concentrations of electron acceptors were
142 the following (mM): Na₂SO₄, 20; Na₂SO₃, 5; Na₂S₂O₃ *5H₂O, 10; NaNO₃, 10;
143 Fe(OH)₃, 100; and S⁰, 300. Amorphous FeOOH suspension (iron gel) was prepared by
144 neutralizing a 0.4 M solution of FeCl₃ to pH 7 by 10 N NaOH as described previously
145 (Lovley & Phillips, 1986). Only elemental sulfur was used as an electron acceptor,
146 which resulted in the production of hydrogen sulfide (15-20 mM). No growth was
147 observed in the absence of sulfur on all tested substrates.

148 The ability of the new archaeon to utilize various substrates was tested by using the
149 liquid medium supplemented with autoclaved or filter-sterilized substrates to a final
150 concentration of 5 g l⁻¹. The substrate utilization was tested by cultivation of strain
151 OGL-20P^T during 1-6 days on different substrates and growth was detected under a
152 microscope and by measurement of hydrogen sulfide. Growth was observed on
153 proteolysis products: peptone, bacto-tryptone, casamino acids, and yeast extract. No
154 growth was observed in the presence of glucose, fructose, maltose, sucrose, *D*-
155 mannitol, glycerol, methanol, ethanol, butyrate, propionate, acetate, formate, lactate,
156 pyruvate, citrate, and separate amino acids (*L*- and *D*- leucine, *L*- and *D*-methionine,
157 *L*- and *D*- histidine HCl, *L*- cysteine, *L*- proline, *L*- lysine, *L*- cystine, glycine, *L*-
158 glutamine, *L*- alanine, *L*- serine, *L*- tyrosine, *L*- phenylalanine, *L*- valine, *L*-
159 isoleucine, *L*- tryptophan, *L*- arginine).

160 End products of sulfur respiration in the liquid phase were determined by HPLC.
161 Separation was done on Aminex HPX-87H (BioRad) column with 5 mM H₂SO₄ as the
162 mobile phase. Gases were measured with a gas chromatograph 3700 (Varian) equipped
163 with Porapak Q column and TCD detector. Nitrogen was used as the gas carrier.
164 Acetate (2.1 mM) and ethanol (3.7 mM) were detected in the liquid phase as minor end
165 products. Hydrogen sulfide (more than 20 mM) and traces of hydrogen and CO₂ were
166 measured in the gas phase during the growth of OGL-20P^T.
167 Antibiotic susceptibility was determined by transferring an exponentially growing
168 culture into the basal medium containing filter-sterilized antibiotics at a concentration
169 of 100 µg ml⁻¹ (chloramphenicol, rifampin) or 250 µg ml⁻¹ (ampicillin, tetracycline,
170 kanamycin, and gentamycin). Before incubation at 83 °C, antibiotic-containing
171 cultures were pre-incubated at 37 °C for 12 h. Strain OGL-20P^T was resistant to
172 ampicillin, gentamycin, kanamycin and chloramphenicol (growth without changes of
173 morphology and motility), but was sensitive to tetracycline and rifampin.
174 Genomic DNA was isolated through a standard phenol / chloroform extraction
175 followed by ethanol precipitation (Sambrook *et al.*, 1989). The G+C content of DNA
176 was determined by HPLC (Mesbah *et al.*, 1989). Details of the procedure were
177 described previously (Hoover *et al.*, 2003). The result reported was the mean of two
178 determinations for each of two degradations of the archaeal DNA. The G+C content
179 of the genomic DNA of strain OGL-20P^T was 57.2 ± 0.2 mol% (mean ± SD, n = 6).
180 The 16S rRNA gene of strain OGL-20P^T, along with a part of 23S rRNA gene and the
181 spacer region, was selectively amplified with the following primers: 5'-
182 TCCGGTTGATCCTGCCGG-3' (forward) and

183 5'-CTTTTCCTGCGGGTACTAAG-3' (reverse). PCR was performed with 30 pmol
184 of each primer in a 50 µl volume, using 2 U ThermalAce DNA polymerase
185 (Invitrogen, USA) in the provided buffer. The thermal cycling profile was as follows:
186 3 min at 95°C initial denaturation, followed by 30 cycles of 45 s denaturation at 95
187 °C, 45 sec annealing at 57 °C and 4 min extension at 72 °C, with a final extension step
188 at 72 °C during 15 min. The amplified fragment was extracted from a 1.5 % agarose
189 gel using the Qiaquick extraction kit (Qiagen, USA), and then subcloned using the
190 Zero Blunt TOPO PCR Cloning kit (Invitrogen, USA). Six clones were sequenced in
191 both directions using the dye terminator AmpliTaq FS cycle sequencing kit (Applied
192 Biosystems, USA) with both vector-based primers and primers specific to 16S
193 internal sequence (designed by us).

194 The 16S rRNA sequence of strain OGL-20P^T was aligned with closely related
195 sequences found in GenBank after a BLAST search (Altschul *et al.*, 1990), using
196 ClustalW (Thompson *et al.* 1994). Pairwise distances were computed with MEGA
197 version 3.1 (Kumar *et al.*, 2004) using the Jukes-Cantor model (Jukes & Cantor,
198 1969). An unrooted phylogenetic tree was constructed with the same MEGA program
199 using the Neighbor-Joining method (Saitou & Nei, 1987).

200 A sequence covering 1885 nucleotides, including most (1452) of the 16S rRNA gene,
201 the tRNA^{Ala} gene and a part of the 23S rRNA gene, was obtained after amplification
202 of strain OGL-20P^T DNA. The 16S rRNA gene sequence corresponds to positions 37-
203 1496 of the *Pyrococcus furiosus* 16S rRNA sequence (accession number U20163)
204 used as a reference. A BLAST search against the Genbank database revealed a high
205 similarity (> 97 %) with sequences from the *Thermococcus* genus. A phylogenetic

206 dendrogram showing the relationship of strain OGL-20P^T to the 11 closest species
207 was constructed, based on 1400 common nucleotide sites (Fig. 2). Pairwise distances
208 between the OGL-20P^T sequence and its closest neighbors were 0.003, 0.006, 0.006
209 and 0.007 for *T. coalescens*, *T. celer*, *T. hydrothermalis* and *T. barossii* respectively
210 based on the same 1400 nucleotide sites. The sequence of the 16S rRNA gene of
211 strain OGL-20P^T was deposited in GenBank under accession number AF394925.
212 Homologies of genomic DNA between the new isolate and the phylogenetically
213 closest *Thermococcus* species were determined as described previously (Pikuta *et al.*,
214 2006). The DNA-DNA hybridization values with labeled DNA from strain OGL-
215 20P^T were as follows: *T. celer* JCM 8558^T: 14 %, *T. barossii* ATCC BAA-1085^T: 17
216 %, *T. hydrothermalis* AL662^T: 16 %, *T. kodakaraensis* ATCC BAA-918^T: 5 %, *T.*
217 *profundus* ATCC 51592^T: 4 %, *T. acidaminovorans* DSM 11906^T: 5 %, *T. stetteri*
218 DSM 5262^T: 4 %, *T. peptonophilus* ATCC 700098^T: 5 %, *T. gorgonarius* ATCC
219 700654^T: 5 %, *T. coalescens* JCM 12540^T: 13 %, and '*T. radiotolerans*' JCM 11826^T:
220 18 %.

221

222 Almost half of the *Thermococcus* species were isolated from deep-sea hydrothermal
223 vents with high pressure conditions (200-350 atmospheres), located in different parts
224 of the world (Kobayashi *et al.*, 1994; Huber *et al.*, 1995; Godfroy *et al.*, 1996; Godfroy
225 *et al.*, 1997; Canganella *et al.*, 1998; Duffaud *et al.*, 1998; Grote *et al.*, 1999). Strain
226 OGL-20P^T was also isolated from a deep-sea ecosystem, characterized by high
227 pressure (230 atmospheres), localized high temperatures (300 to 400 °C within the
228 Black Smoker vents), and very high thermal gradients, (temperature drops to 2 °C a

229 few centimeters away from the “chimney”). As the “smoke” condenses above the
230 vent, the precipitated minerals are also spread around the nearby ocean floor; black
231 color pyrites (FeS) surrounding Black Smokers is a result of interaction of sulfide
232 with iron; orange color is a result of a trivalent iron (Fe⁺³) appearance. Unpigmented
233 invertebrates (shrimps, crabs, and worms) represent multicellular organisms in the
234 ecosystem. Their energy source is partially provided by the metabolism of
235 microorganisms (in our laboratory the cells with morphology similar to the new
236 archaeon were found in the intestines of shrimps with a strong smell of sulfur.)

237 Strain OGL-20P^T is a hyperthermophilic, heterotrophic, sulfur-dependent, coccoid
238 archaeon inhabiting a deep-sea hydrothermal system in the Mid-Atlantic Ridge. In
239 line with those properties, comparison of the 16S rRNA gene places the strain in a
240 clade of the euryarchaeotic order, the order *Thermococcales*, and most related to the
241 genus *Thermococcus*. Currently, the genus *Thermococcus* contains 25 validly
242 published species, which are separated into two major clades represented by *T. celer*
243 and *T. litoralis*, and two independent lineages of *T. barophilus* and *T. atlanticus*. The
244 separation of the two major clades is also supported by the DNA base composition.
245 The strain OGL-20P^T is included in the clade represented by *T. celer*. Comparison of
246 strain OGL-20P^T with closest neighbors on the phylogenetic tree showed a 16S rRNA
247 sequence difference of less than 1 %. However, the DNA-DNA- hybridization
248 showed less than 20 % similarity with them. Phenotypic and genotypic differences
249 between strain OGL-20P^T and the closest species are shown in comparative table1.

250 On the basis of comparative data about morphology, physiology and genomic
251 characteristics we conclude that strain OGL-20P^T represents a separate taxon on the

252 species level. The name *Thermococcus thioeducens* sp. nov., is proposed for the new
253 species.

254

255 **Description of *Thermococcus thioeducens* sp. nov.**

256

257 *Thermococcus thioeducens* (thi.o.re.du'cens. Gr. n. *thion* sulfur, L. part. adj.

258 *reducens* reducing, N.L. part. adj. *thioeducens* reducing sulfur).

259

260 Cells are irregular cocci with 0.7- 1.8 µm diameter, motile by single flagellum.

261 Heterotroph, strict anaerobe. Obligately dependent from elemental sulfur. Catalase

262 negative. Grows on peptone, bacto-tryptone, casamino acids, yeast extract as electron

263 donors. No growth on *D*-glucose, fructose, maltose, sucrose, *D*-mannitol, glycerol,

264 methanol, ethanol, butyrate, propionate, acetate, formate, lactate, pyruvate, citrate and

265 amino acids. Thiosulfate, sulfite, sulfate, iron (III) or nitrate cannot support growth as

266 electron acceptors. Cells are hyperthermophiles growing between 55 °C and 94 °C

267 with optimum at 83- 85 °C, and pH range 5.0 - 8.5 (optimum 7.0), and with NaCl

268 concentration (w/v) range 1 - 5 % (optimum 3 %) The doubling time is 30 min. The

269 main end product of growth with peptone and sulfur is H₂S (more than 20 mM);

270 minor end products are: CO₂, H₂ (0.05 mM), acetate (2 mM), ethanol (3.7 mM).

271 Sensitive to tetracycline and rifampin. The G+C content of DNA is 52.9 mol%

272 (HPLC).

273 Source of isolation: deep sea “Black Smoker” chimney debris in mud at Rainbow
274 hydrothermal vent site at 2,300 meters depth in Atlantic Ocean off the coast of the
275 Azores.
276 Type strain: *Thermococcus thioreducens* OGL-20P^T (= JCM 12859^T = DSM 14981^T
277 = ATCC BAA-394^T).

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279

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290

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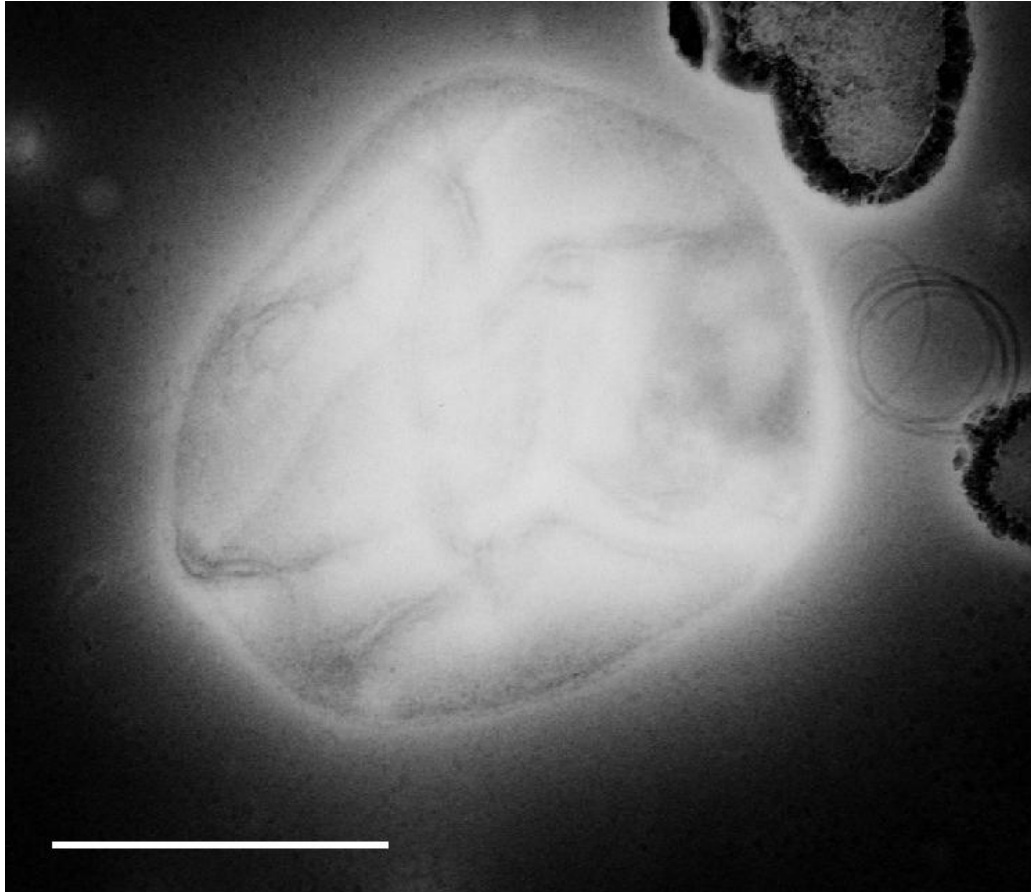
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433

FIGURES



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436 **Fig. 1.** TEM image by negative staining of cell of strain OGL-20P^T with single coiled
437 flagellum. Bar = 0.5 μ m.

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440

441 **Fig. 2.** Unrooted phylogenetic tree indicating the position of strain OGL-20P^T among
442 closest taxa. Numbers at the nodes are bootstrap values obtained from 1000
443 replicates. Nucleotide sequence accession numbers are shown in parentheses. Scale
444 bar indicates 1 inferred nucleotide substitution per 1000 nucleotides.

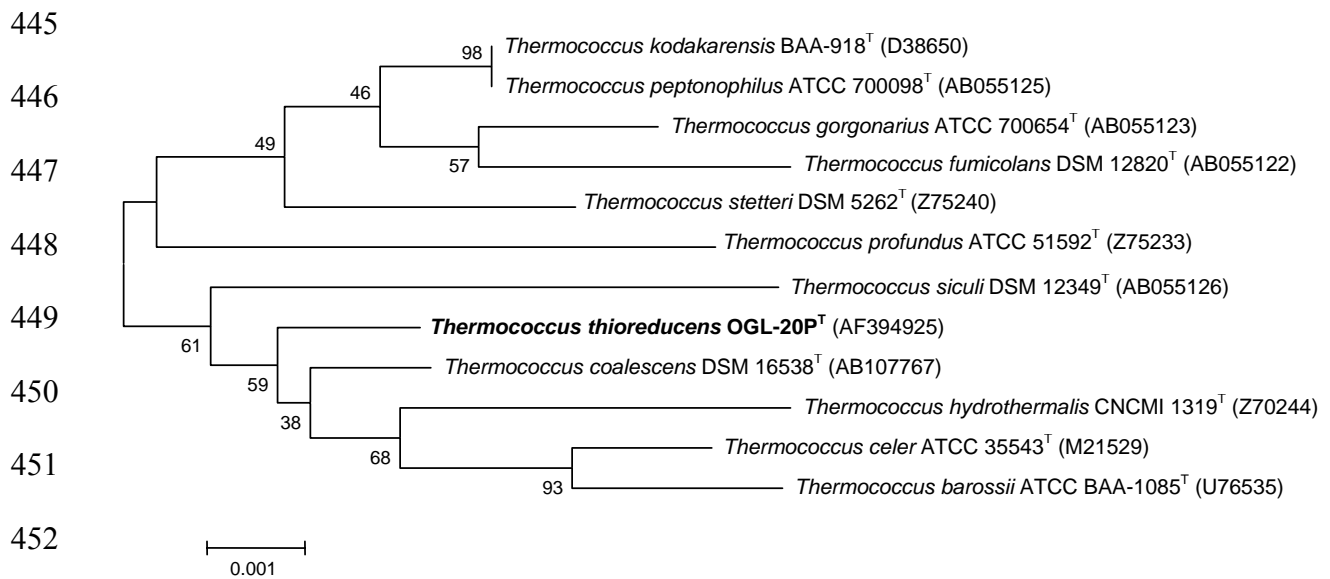


Table 1. Comparative table for strain OGL-20P^T and phylogenetically closest species.

Species: Features:	<i>T. thioaeruginosa</i> OGL-20P ^T (present work)	<i>T. coalescens</i> JCM 12540 ^T (Kuwabara <i>et al.</i> , 2005)	<i>T. cedar</i> ATCC 35543 ^T (Zillig <i>et al.</i> , 1983)	<i>T. barossii</i> DSM 9535 ^T (Daufland <i>et al.</i> , 1998)	<i>T. hydrothermalis</i> AL662 ^T (Godfrey <i>et al.</i> , 1997)	<i>T. kodakarensis</i> ATCC BAA-918 ^T (Aonomi <i>et al.</i> , 2004)	<i>T. profundus</i> ATCC 51592 ^T (Kobayashi <i>et al.</i> , 1994)	<i>T. acidaminovorans</i> DSM 11906 ^T (Dirmeier <i>et al.</i> , 1998)	<i>T. stetteri</i> DSM 5262 ^T (Miroshnichenko <i>et al.</i> , 1989)	<i>T. pentanophilus</i> ATCC 700098 ^T (Gonzalez <i>et al.</i> , 1995)	<i>T. gorgonaensis</i> ATCC 700654 ^T (Miroshnichenko <i>et al.</i> , 1998)
G+C, mol%	52.9	53.9	56.6	60.0	58.0	52	52.5	49	50	52	50.6
Motility/ Flagellation	motile/ monotrichous	motile/tuft of flagella	motile/polar polytrichous	non-motile	motile/ monotrichous	motile/ polar tuft of flagella	motile/polar tuft of flagella	motile/tuft of flagella	non-motile	motile/ facultative	motile/polar flagella
S ⁰ requirement	obligate	⁵	facultative	obligate	facultative	facultative	obligate	facultative	obligate	facultative	obligate
pH opt. (range)	7.0 (5.0-8.5)	6.5 (5.2-8.7)	5.8	6.5-7.5 (3-9)	6.0	6.5 (5-9)	4.5-8.5	9.0 (5.0-9.5)	6.5 (5.7-7.2)	6 (4-8)	6.5-7.2 (5.8-8.5)
NaCl, % opt.(range)	3.0 (1.0-5.0)	2.5 (1.5-4.5)	3.8-4.0 (1-4)	2.0 (1.0-4.0)	2-3	3 (1-5)	2-4 (1-6)	2-3 (1-6)	2.5 (1-4)	3 (1-5)	2.0-3.5 (1-5)
T, °C opt. (range)	83-85 (55-95)	87 (57-90)	88 (50-94)	82.5 (60-94)	85 (55-100)	85 (60-100)	80 (50-90)	85 (56-93)	76 (55-94)	85-90 (60-100)	80-88 (68-95)
Substrates: Protolysis products^{1,2} Starch³ Pyruvate	+ - -	+ - ND	+ ND ND	+ ND ND	+ - (+)	+ + +	+ + +	+ - ND	+ + ND	+ ND -	+ - (+)
End products:											
H ₂	+	ND	+	+	+	+	+	ND	ND	-	+
CO	+	ND	-	+	+	ND	+	ND	ND	+	+
CO ₂	+	ND	+	+	+	ND	+	+	+	+	+
Acetate	+	ND	ND	ND	+	ND	ND	ND	+	ND	+
Laetate	-	ND	ND	ND	+	ND	ND	ND	ND	ND	ND
Iso-butyrate	-	ND	ND	ND	+	ND	ND	ND	+	ND	ND
Propionate	-	ND	ND	ND	+	ND	ND	ND	-	ND	+
Methyl-propionate	-	ND	ND	ND	+	ND	ND	ND	-	ND	-
Iso-valerate	-	ND	ND	ND	-	ND	ND	ND	+	ND	+
t _D , min	30	ND	50	33	90	ND	50	120	72	25	ND
Genome size, Dalton	1.23 x 10 ⁹	ND	1.24 x 10 ⁹	1.18 x 10 ⁹	ND	ND	ND	ND	ND	ND	ND
Rifampin resistance	-	-	+	-	+	ND	-	ND	-	-	-

^{1,2,3} - positive test; ^{4,5} - negative test; ⁶(+) - weak growth; ND - no data. Genome size was detected as described previously (Hoover *et al.*, 2003). ⁵ - stimulatory only.

⁷ - all strains hydrolyze casein & starch, but not chitin; ⁸ - hydrolysed proteins or peptides (peptone, yeast extract, meat extract etc.); ⁹ - produces 2-methyl-propionate, 3-methyl-ithio-propionate & propanoate.