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1 ***Thermotomaculum hydrothermale* gen. nov., sp. nov., a novel heterotrophic**
2 **thermophile within the phylum *Acidobacteria* from a deep-sea hydrothermal vent**
3 **chimney in the Southern Okinawa Trough**

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27 **Abstract**

28 A novel heterotrophic, thermophilic bacterium, designated strain AC55^T, was isolated
29 from a deep-sea hydrothermal vent chimney at the Hatoma Knoll in the Okinawa
30 Trough, Japan. Cells of strain AC55^T were non-motile, long rods (2.0-6.8 μm long and
31 0.3-0.6 μm wide). The strain was an obligatory anaerobic heterotroph capable of
32 fermentative growth on complex proteinaceous substances. Elemental sulfur was
33 reduced to hydrogen sulfide but did not stimulate growth. Growth was observed
34 between 37 and 60 °C (optimum 55 °C), pH 5.5 and 8.5 (optimum pH 6.6), and in the
35 presence of 1.5-4.5 % (w/v) NaCl (optimum 2.5 %, w/v). Menaquinone-7 and -8 were
36 the major respiratory quinones. The G + C content of the genomic DNA from strain
37 AC55^T was 51.6 mol%. The 16S rRNA gene sequence analysis revealed that strain
38 AC55^T was the first cultivated representative of *Acidobacteria* subdivision 10. Based on
39 the physiological and phylogenetic features of the novel isolate, the genus name
40 *Thermotomaculum* gen. nov. is proposed, with *Thermotomaculum hydrothermale* sp.
41 nov. as the type species. The type strain is AC55^T (= JCM 17643^T = DSM 24660^T =
42 NBRC 107904^T).

43

44 **Keywords** *Acidobacteria* • Deep-sea hydrothermal vent • Thermophile • Fermentation

45

45 **Introduction**

46 The phylum *Acidobacteria* comprises twenty-six distinct subdivisions (Barns et al.
47 2007). Only subdivisions 1, 3 and 8 have genera with validly published names.
48 Subdivision 1 includes the genera *Acidobacterium* (Kishimoto et al. 1991), *Terriglobus*
49 (Eichorst et al. 2007; Männistö et al. 2010), *Edaphobacter* (Koch et al. 2008),
50 *Granulicella* (Pankratov and Dedysh 2010), *Acidicapsa* (Kulichevskaya et al. 2011),
51 *Telmatobacter* (Pankratov et al. 2011), and *Bryocella* (Dedysh et al. 2011). The only
52 described genus in subdivision 3 is *Bryobacter* (Kulichevskaya et al. 2010), while
53 subdivision 8 includes the genera *Holophaga* (Liesack et al. 1994), *Geothrix* (Coates et
54 al. 1999), and *Acanthopleuribacter* (Fukunaga et al. 2008). All the above listed,
55 taxonomically characterized acidobacteria are mesophiles. Strain K22 isolated from a
56 New Zealand hot spring is a member of subdivision 4 and the only thermophilic
57 acidobacterium growing at temperatures up to 75 °C (Stott et al. 2008). In addition,
58 subdivision 4 includes an aerobic phototrophic thermophile, ‘*Candidatus*
59 *Chloracidobacterium thermophilum*’ (Bryant et al. 2007). Whole genome sequences are
60 currently available for *Acidobacterium capsulatum* DSM11244^T (accession no.
61 NC_012483), *Terriglobus saanensis* SP1PR4^T (accession no. NC_014963),
62 *Granulicella tundricola* MP5ACTX9^T (accession no. NC_015064), ‘*Koribacter*

63 versatilis' strain Ellin345 (subdivision 1; NC_008009), 'Solibacter usitatus' strain
64 Ellin6076 (subdivision 3; NC_008536) (Ward et al. 2009) and '*Candidatus*
65 *Chloracidobacterium thermophilum*' (Garcia Costas et al. 2011).

66 Members of the phylum *Acidobacteria* inhabit a wide variety of environments
67 (Pankratov and Dedysh 2010). They have been detected in soil (Ludwig et al. 1997; Sait
68 et al. 2002; Barns et al. 1999, 2007), hot springs (Barns et al. 1999; Hugenholtz et al.
69 1998; Bryant et al. 2007), acidic mining lakes (Kleinsteuber et al. 2007; Kampe et al.
70 2010), caves (Zimmermann et al. 2005; Meisinger et al. 2007), shallow submarine vents
71 (Sievert et al. 2000), and deep-sea hydrothermal fields (López-García et al. 2003;
72 Brazelton et al. 2006; Nunoura and Takai 2009; Nunoura et al. 2010). In this study, a
73 novel strain of thermophilic acidobacteria is described that was isolated from a deep-sea
74 hydrothermal field.

75

76 **Materials and methods**

77 **Sample collection** A sample from a deep-sea hydrothermal vent chimney was
78 obtained from the Hatoma Knoll (24°51'N, 123°50'E) in the Southern Okinawa Trough
79 at a depth of 1470 m by means of a ROV Hyper Dolphin in July 2008. The chimney
80 portions were broken by a manipulator of the ROV at the 189-1 vent and directly

81 dropped into a sample box. Immediately after the recovery of chimney sample onboard,
82 a relatively large piece of structure was divided into exterior surface and vent orifice
83 portions, and suspended in sterilized seawater in the presence of 0.05 % (w/v)
84 neutralized sodium sulfide in a 100 ml glass bottle (Schott Glaswerke). The bottle was
85 tightly sealed with a butyl rubber stopper under a gas phase of 100 % N₂ (200 kPa).

86 **Cultivation** The suspended slurry was used to inoculate MMJSO medium
87 (Nunoura et al. 2007), which was further incubated at 55 °C. MMJSO medium
88 contained 0.02 % (w/v) yeast extract, 0.05 % (w/v) pyruvate, 0.05 % (w/v) lactate,
89 0.1 % (w/v) NaHCO₃, 0.05 % (w/v) ascorbic acid, and 1 mg resazurin per liter of MJ
90 synthetic seawater (Sako et al. 1996) under a gas mixture of H₂:CO₂ (80:20) (200kPa).
91 MJ synthetic seawater is composed of (per liter) NaCl, 30 g; MgCl₂·6H₂O, 4.18 g;
92 MgSO₄·7H₂O, 3.4 g; KCl, 0.33 g; NH₄Cl, 0.25 g; K₂HPO₄, 0.14 g; CaCl₂·2H₂O, 0.14 g;
93 and trace mineral solution, 10 ml. Trace mineral solution contains (per liter)
94 nitrilotriacetic acid, 1.5 g; MgSO₄·7H₂O, 3.0 g; MnSO₄·2H₂O, 0.5 g; NaCl, 1.0 g;
95 FeSO₄·7H₂O, 0.1 g; CoSO₄·7H₂O, 0.18 g; CaCl₂·2H₂O, 0.1 g; ZnSO₄·7H₂O, 0.18 g;
96 CuSO₄·5H₂O, 0.01 g; KAl(SO₄)₂·12H₂O, 0.02 g; H₃BO₃, 0.01 g; Na₂MoO₄·2H₂O, 0.01
97 g; NiCl₂·6H₂O, 0.025 g; and Na₂SeO₃·5H₂O, 0.3 mg.

98 The presence or absence of cell growth was determined by microscopic observation.
99 In order to obtain consistent growth, gas phase of the MMJSO medium was changed to
100 a gas mixture of N₂:CO₂ (80:20) (200kPa). To obtain a pure culture, a
101 dilution-to-extinction method was employed at 55 °C and repeated at least five times
102 (Baross 1995). Purity was confirmed routinely by microscopic observation and by
103 repeated partial sequencing of the 16S rRNA gene using several PCR primers.

104 The isolate was routinely cultivated in MMJYP2 medium, which contains 0.4 %
105 (w/v) yeast extract, 0.4 % (w/v) tryptone peptone, 0.1 % (w/v) NaHCO₃ and 0.05 %
106 (w/v) Na₂S in modified MJ synthetic seawater (Nakagawa and Takai 2006). Modified
107 MJ synthetic seawater is composed (per liter) of NaCl, 25 g; MgCl₂·6H₂O, 4.2 g;
108 MgSO₄·7H₂O, 3.4 g; KCl, 0.5 g; NH₄Cl, 0.25 g; K₂HPO₄, 0.14 g; CaCl₂·2H₂O, 0.7 g.
109 To prepare MMJYP2 medium, all components other than Na₂S and NaHCO₃ were
110 dissolved. After autoclaving, a concentrated and filter-sterilized solution of NaHCO₃,
111 and neutralized Na₂S solution (pH7.5) (sterilized by autoclaving) were added to the
112 medium under gas purging of 80 % N₂ and 20 % CO₂. The tubes were then tightly
113 sealed with butyl rubber stoppers under a gas phase of 80 % N₂ + 20 % CO₂ (350 kPa).
114 No growth was observed when both NaHCO₃ and CO₂ were eliminated from the
115 medium.

116 **Light and electron microscopy** Cells were routinely observed by using a ZEISS
117 Axiophot microscope (Carl Zeiss). Transmission electron micrographs of negatively
118 strained and thin section cells grown in MMJYP2 medium at 55 °C in the
119 late-exponential phase were obtained as described by Zillig et al. (1990).

120 **Measurement of growth** Growth of novel isolate was determined by direct cell
121 counts, after staining with 6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980).
122 To determine temperature, pH and NaCl ranges for growth, duplicate cultures were
123 grown in 15 ml test tubes containing 3 ml medium in an incubator. Effects of pH and
124 NaCl concentration on the growth of isolate were determined at 55 °C. NaCl
125 requirements were determined with varying concentrations of NaCl in MMJYP2
126 medium from 0.5 to 5.5 % (w/v). When the pH optimum was examined, pH of the
127 medium was readjusted immediately before inoculation with H₂SO₄ or NaOH by using
128 a compact pH meter (Horiba AS-212) at 55 °C. The pH was found to be stable during
129 the cultivation period.

130 In an attempt to examine the ability of respiratory growth, possible electron
131 acceptors were added to MMJYP2 medium at final concentrations of 0.1 % (w/v,
132 Na₂S₂O₃·5H₂O, NaNO₃, ferric citrate, and Na₂SO₄), 0.1 % (v/v, O₂), 0.01-0.1 % (w/v,
133 Na₂SO₃ and NaNO₂) or 1 % (w/v, S⁰). O₂ was provided by injecting a defined volume

134 of O₂ (0.1-10 %, v/v) into the culture tubes as previously described (Nakagawa et al.
135 2003). The production of hydrogen sulfide was detected by using lead acetate solution.

136 In an attempt to find organic substrates that could support the growth of isolate,
137 experiments were conducted in which the yeast extract and tryptone peptone in
138 MMJYP2 medium were replaced with other organic materials as potential substrates
139 under a gas phase of N₂:CO₂ (80:20, 350 kPa). Each of the following substrates was
140 added at concentrations of 0.01 % or 0.1 % (w/v): L-cystine, L-phenylalanine, L-proline,
141 Casamino acids, (+)-D-glucose, lactose, maltose, chitin, starch, cellulose, formate,
142 formaldehyde, acetate, citrate, pyruvate, propionate, methanol, tryptone peptone and
143 yeast extract (Difco). Products of fermentative growth were identified with F-kit (Roche
144 Applied Science, USA) and H₂ detector tube (Gastec, Japan). Chemolithoautotrophic
145 growth was examined as described in Nakagawa et al. (2005).

146 **Lipid components** Respiratory lipoquinones and polar lipids were extracted from
147 freeze-dried cells following Minnikin et al. (1984). Cells grown in MMJYP2 medium at
148 55 °C in the late-exponential phase of growth were used. Respiratory lipoquinones were
149 dissolved in petroleum ether and applied to TLC plates (silica gel). After development
150 with hexane-benzene-chloroform (5:2:1, v/v) separated components were detected at
151 UV-254 nm. Standards of vitamin K₁ and ubiquinone-50 (coenzyme Q₁₀) were used to

152 locate bands corresponding to menaquinone and ubiquinone, respectively.

153 UV-absorbing bands were removed from the plates and further analyzed by using a

154 Shimadzu HPLC with a reverse phase Kinetex C18 column and methanol-isopropanol

155 (3:1, v/v) as the mobile phase at 1ml/min at 37 °C and were detected at 269 nm

156 (Tamaoka et al. 1983). Polar lipids were separated by two-dimensional silica gel TLC as

157 described in Pankratov et al. (2011). The plates were sprayed with molybdophosphoric

158 acid (total lipids), molybdenum blue (phospholipids), ninhydrin (free amino groups) and

159 α -naphthol reagents (glycolipids) and Dragendorff reagent (quaternary nitrogen). The

160 standards of phospholipids (Sigma, USA) were used for diagram disposition of

161 phospholipids during comparative analysis.

162 For fatty acid analysis, lyophilized cells were placed in a Teflon-lined, screw-capped

163 tube containing 1ml of anhydrous methanolic HCl and heated at 100 °C for 3 h. The

164 extraction and analysis of fatty acid methyl esters have been described previously

165 (Komagata and Suzuki 1987). For comparative purposes, type strains of

166 *Acidobacterium capsulatum* (JCM7670), *Staphylococcus epidermidis* (JCM2414),

167 *Streptomyces olivaceus* (JCM4066), and *Leifsonia shinshuensis* (JCM10591) were used.

168 **DNA base composition** Genomic DNA was isolated by a standard

169 phenol/chloroform extraction followed by ethanol precipitation (Sambrook et al. 1989).

170 The G + C content was determined by direct analysis of deoxyribonucleosides by HPLC
171 (Tamaoka and Komagata 1984).

172 **16S rRNA gene analysis** The 16S rRNA gene was amplified by PCR using
173 primers Eubac 27F and 1492R (Lane 1991). Sequence of the PCR product (1,412 bp)
174 was determined directly in both strands using the dideoxynucleotide chain termination
175 method. The rRNA gene sequence was applied to sequence similarity analysis with
176 databases by the BLAST search algorithm (Altschul et al. 1997). In order to determine
177 the phylogenetic position of the isolate, the sequence was aligned with a subset of 16S
178 rRNA gene sequences by ARB software (Ludwig et al. 2004). Resulting alignment was
179 verified against known secondary regions, and only unambiguously aligned nucleotide
180 positions (1126 bases) were used for phylogenetic analyses with PAUP* 4.0 beta 10
181 (Swofford 2000). Phylogenetic tree was inferred by using neighbor-joining analysis
182 (Saitou and Nei 1987) with the Jukes and Cantor correction (Jukes and Cantor 1969).
183 Bootstrap analysis was used for 100 or 1000 replications to provide confidence
184 estimates for the phylogenetic tree topologies.

185

186 **Results and discussion**

187 **Enrichment and purification** Microbial growth was only observed from the
188 exterior surface of chimney structure at 55 °C. The pure culture obtained was
189 designated strain AC55^T and investigated in detail. Cells of strain AC55^T are long
190 rod-shaped, observed singly, but can also occur as a group of 3-4 cells in a chain-like
191 structure (Fig. 1a) or as aggregates of up to 40-50 cells (Supplementary Fig. S1). No
192 flagellum was observed (Fig. 1a). Electron micrographs of thin sections showed that the
193 isolate had an envelope consisting of a cytoplasmic membrane and outer membrane (Fig.
194 1b). No sporulation was apparent under any laboratory conditions.

195 **Growth characteristics** The isolate grew over the temperature range of about
196 37-60 °C, showing optimum growth at 55 °C. The generation time and maximum cell
197 yield at 55 °C, 2.5 % (w/v) NaCl, pH 6.0, were about 3 h and approximately 4.0×10^7
198 cells/ml, respectively. No growth was observed at 30 °C or 65 °C (Supplementary Fig.
199 S2a). The isolate grew in the concentration range of about 1.5 to 4.5 % (w/v) NaCl,
200 showing optimum growth at approximately 2.5 % (w/v) NaCl (Supplementary Fig. S2b).
201 The isolate grew over the pH range of about pH 5.5-8.5, showing optimum growth at
202 pH 6.6. No growth was detected at pH 5.0 or pH 8.5 (Supplementary Fig. S2c).

203 **Nutrition** The isolate was able to utilize 0.1 % (w/v) yeast extract and 0.1 % (w/v)
204 tryptone peptone as sole energy and carbon sources. Acetate was detected as the product

205 of fermentative growth. H₂ formation was not detected (detection limit ≥ 0.5 %, v/v).
206 L-cystine, L-phenylalanine, L-proline, Casamino acids, (+)-D-glucose, lactose, maltose,
207 chitin, starch, cellulose, formate, formaldehyde, acetate, citrate, pyruvate, propionate,
208 methanol, 0.01 % (w/v) tryptone peptone and yeast extract did not support the growth.

209 The growth of strain AC55^T was inhibited by the addition of 0.1 % (w/v) Na₂SO₃,
210 0.1 % (w/v) ferric citrate, and 0.01-0.1 % (w/v) NaNO₂. In other cases, possible electron
211 acceptors used in this study resulted in no significant differences in growth rate or in
212 maximal yield, although S⁰ was reduced to hydrogen sulfide.

213 **Lipid components** Strain AC55^T contained menaquinone-8 (MK-8; 85.6 %) and
214 -7 (MK-7; 14.4 %) as the predominant isoprenoid quinones. Members of the phylum
215 *Acidobacteria* subdivision 1 also contained MK-8 as the predominant isoprenoid
216 quinones but not MK-7 (Table 1). As shown by TLC, strain AC55^T possesses
217 phosphatidylethanolamine, unidentified aminophospholipids, and unidentified
218 phospholipids (Supplementary Fig. S3). The cellular fatty acids of strain AC55^T were
219 C_{17:0} (66.7 %), C_{15:0} (26.2 %), C_{14:0}-OH (4.6 %), and C_{16:0} (2.5 %). The dominance of
220 odd-chain fatty acids is a shared feature among acidobacteria.

221 **DNA base composition** The G + C content of genomic DNA from strain AC55^T
222 was 51.6 mol% (Table 1).

223 **Phylogenetic analysis** The 16S rRNA gene sequence of strain AC55^T was applied
224 to sequence similarity analysis with databases by the BLAST search algorithm (Altschul
225 et al. 1997). Among the species with validly published names, *Holophaga foetida*
226 (85 %) and *Geothrix fermentans* (84 %) were the closest relatives of the isolate. The
227 phylogenetic tree indicated that strain AC55^T was the first cultivated member of
228 subdivision 10 within the phylum *Acidobacteria* (Fig. 2). This subdivision contained
229 environmental clone sequences retrieved from various deep-sea habitats, including
230 hydrothermal sediments (López-García et al. 2003) and basaltic lavas (Santelli et al.
231 2008) (Fig. 2).

232 **Comparison with related genera** A number of fermentative thermophiles and
233 hyperthermophiles, such as members of the *Thermococcales* and *Thermotogales*, have
234 been found in deep-sea hydrothermal environments (Takai et al. 2006; Nakagawa and
235 Takai 2008). Recently, additional lineages of deep-sea thermophilic fermenters have
236 been characterized (Reysenbach et al. 2006; Imachi et al. 2008), suggesting the diversity
237 of fermenters in deep-sea vents might still be underestimated. Although differences in
238 their growth strategies *in-situ* remain to be studied, strain AC55^T is unique in that its
239 growth is not stimulated by elemental sulfur.

240 Strain AC55^T is the first isolate within the phylum *Acidobacteria* from deep-sea
241 hydrothermal environments. Although acidobacteria represent an ubiquitous microbial
242 group (Barns et al. 2007), they have been rarely found in deep-sea (López-García et al.
243 2003). All previously described members of the phylum *Acidobacteria* are mesophilic
244 heterotrophs mostly from terrestrial environments. Considering thermophilic
245 acidobacteria was also isolated from terrestrial hot spring (Stott et al. 2008), this group
246 of bacteria has important roles in elemental cycles not only in temperate but in hot
247 environments. On the basis of these results, a new genus, *Thermotomaculum* gen. nov.,
248 is proposed. The type species is *Thermotomaculum hydrothermale* gen. nov., sp. nov.,
249 of which the type strain is AC55^T (= JCM 17643^T = DSM 24660^T = NBRC 107904^T).

250 **Description of *Thermotomaculum* gen. nov.** *Thermotomaculum*

251 (Ther.mo.to.ma'cu.lum. Gr. fem. n. *thermê*, heat; L. neut. n. *tomaculum*, a kind of
252 sausage; N.L. neut. n. *Thermotomaculum*, a sausage-shaped thermophile). Non-motile
253 rods that stain Gram-negative. Anaerobic. Thermophilic. Heterotrophic. Growth by
254 fermentation. Major cellular fatty acids are C_{17:0} and C_{15:0}. Major quinones are
255 menaquinone-7 and -8. Major polar lipids are phosphatidylethanolamine, unidentified
256 aminophospholipids, and unidentified phospholipids. Members of the genus
257 *Thermotomaculum* occur at deep-sea hydrothermal fields. The type species is

258 *Thermotomaculum hydrothermale*.

259 **Description of *Thermotomaculum hydrothermale* sp. nov.** *Thermotomaculum*

260 *hydrothermale* (hy.dro.ther.ma'le. N.L. neut. adj. *hydrothermale*, pertaining to a

261 hydrothermal vent). Cells are non-motile, with a mean length of 2.0-6.8 μm and width

262 of approximately 0.3-0.6 μm . The temperature range for growth is 37-60 $^{\circ}\text{C}$ (optimum

263 55 $^{\circ}\text{C}$). The pH range for growth is 5.5-8.5 (optimum 6.6). NaCl in the concentration

264 range for growth is 15-45 g/l (optimum 25 g/l). Fermentative growth occurs with yeast

265 extract, tryptone peptone as the sole carbon and energy source. The major cellular fatty

266 acids are C_{17:0} and C_{15:0}. The G + C content of the genomic DNA is 51.6 mol%. Isolated

267 from a deep-sea hydrothermal vent in the Southern Okinawa Trough, Japan. The type

268 strain is AC55^T (=JCM 17643^T =DSM 24660^T = NBRC 107904^T). The

269 DDBJ/EMBL/GenBank accession number for the 16S rRNA gene of strain AC55^T is

270 AB612241.

271

272

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463

464 **Table 1.** Comparison of major characteristics of strain AC55^T with those of other members of the phylum *Acidobacteria*.
 465 Strains: 1, *Thermotomaculum hydrothermale* AC55^T; 2, *Acidobacterium capsulatum* 161^T (Kishimoto et al. 1991); 3, *Edaphobacter* spp.
 466 (Koch et al. 2008); 4, *Acidicapsa* spp. (Kulichevskaya et al. 2011); 5, *Granulicella* spp. (Pankratov and Dedysch 2010); 6, *Terriglobus*
 467 spp. (Eichorst et al. 2007; Männistö et al. 2010); 7, *Bryocella elongata* SN10^T (Dedysch et al. 2011); 8, *Telmatobacter bradus* TPB6017^T
 468 (Pankratov et al. 2011); 9, *Bryobacter aggregatus* MPL3^T (Kulichevskaya et al. 2010); 10, *Acanthopleuribacter pedis* FYK2218^T
 469 (Fukunaga et al. 2008); 11, *Geothrix fermentans* H-5^T (Coates et al. 1999); 12, *Holophaga foetida* TMBS4^T (Liesack et al. 1994).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Subdivision	10	1	1	1	1	1	1	1	3	8	8	8
Source of isolation	Deep-sea hydrothermal vent	Acid mine drainage	Alpine and forest soil	Peat and wood	Peat and <i>Cladonia</i>	Soil and termite hindgut	Peat	Peat	Peat	Chiton	Petroleum contaminated aquifer	Freshwater mud
Motility	-	+	+/-	-	-	-	-	+	-	+	-	ND
Aerobic or anaerobic	Anaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Anaerobic	Aerobic	Aerobic	Anaerobic	Anaerobic
Temp. range (°C)	37-60	20-37	15-37	10-33	2-33	4-30	6-32	4-35	4-33	15-30	ND	10-35
Temp. optimum (°C)	55	30	30	22-28	18-22	25	20-24	20-28	22-28	30	35	28-32
pH range	5.5-8.0	3.0-6.0	4.0-7.0	3.5-7.3	3.0-7.5	5.0-7.0	3.2-6.6	3.0-7.5	4.5-7.2	4-9	ND	5.5-8.0
pH optimum	6.6	ND	5.5	4.0-5.5	3.8-4.5	6.0	4.7-5.2	4.5-5.0	5.5-6.5	7-8	ND	6.8-7.5
NaCl range (%)	1.5-4.5	ND	ND	< 2.0	≤ 3.5	ND	< 3.0	< 0.1	≤ 1.5	ND*	ND	ND
NaCl optimum (%)	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND*	ND	ND
Major quinone	MK-7, MK-8	MK-8	ND	MK-8	MK-8	ND	MK-8	MK-8	MK-9, MK-10	MK-6, MK-7	ND	ND
GC content (mol%)	51.6	59.7-60.8	55.8-56.9	51.7-54.1	57.3-59.3	58.1-59.8	60.7	57.6	55.5-56.5	56.7	ND	62.5

470 ND, not determined

471 *Growth was observed on R2A agar containing 50-150 % artificial seawater (optimum 70-120 % ASW).

472 **FIGURE LEGENDS**

473

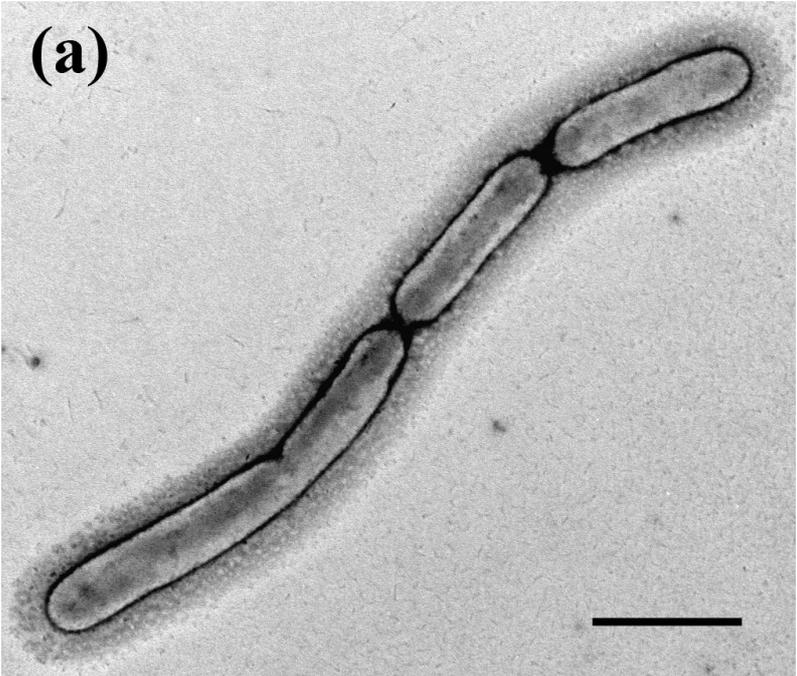
474 **Fig. 1.** Electron micrograph of a negatively stained cell (a) and thin section (b) of strain
475 AC55^T. Arrowhead, cytoplasmic membrane; arrow, outer membrane. Bars, 2.0 μm (a)
476 and 1.0 μm (b).

477

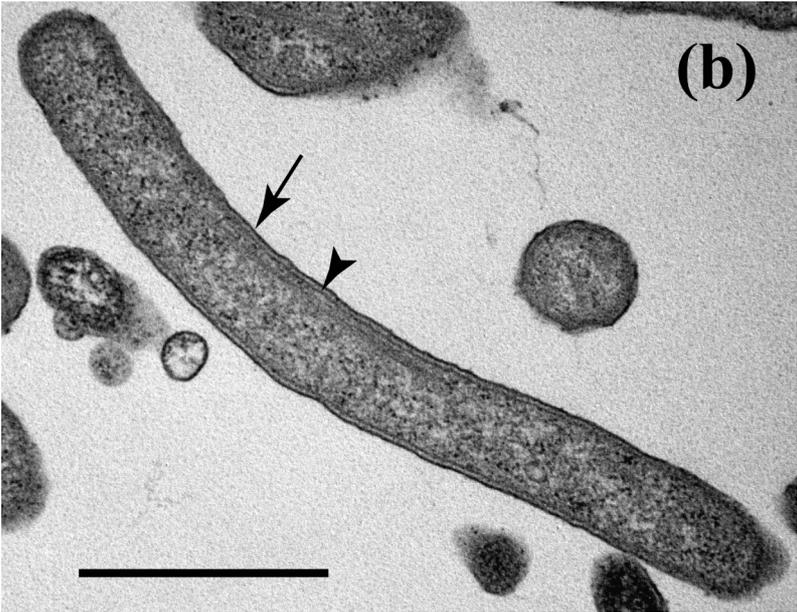
478 **Fig. 2.** Neighbor-joining phylogenetic tree based on 990 aligned positions of the 16S
479 rRNA gene sequence. Bootstrap analyses (100 replications for the maximum-likelihood
480 and 1000 replications for the neighbour-joining) were used to obtain confidence
481 estimates for the tree topology. Branch points conserved with bootstrap values of >
482 75 % (solid circles) and with bootstrap values of > 50 % (gray circles) with the both
483 neighbour-joining and maximum-likelihood methods are indicated. The accession
484 numbers for sequences are given in parentheses. The scale bar represents the expected
485 number of changes per nucleotide position.

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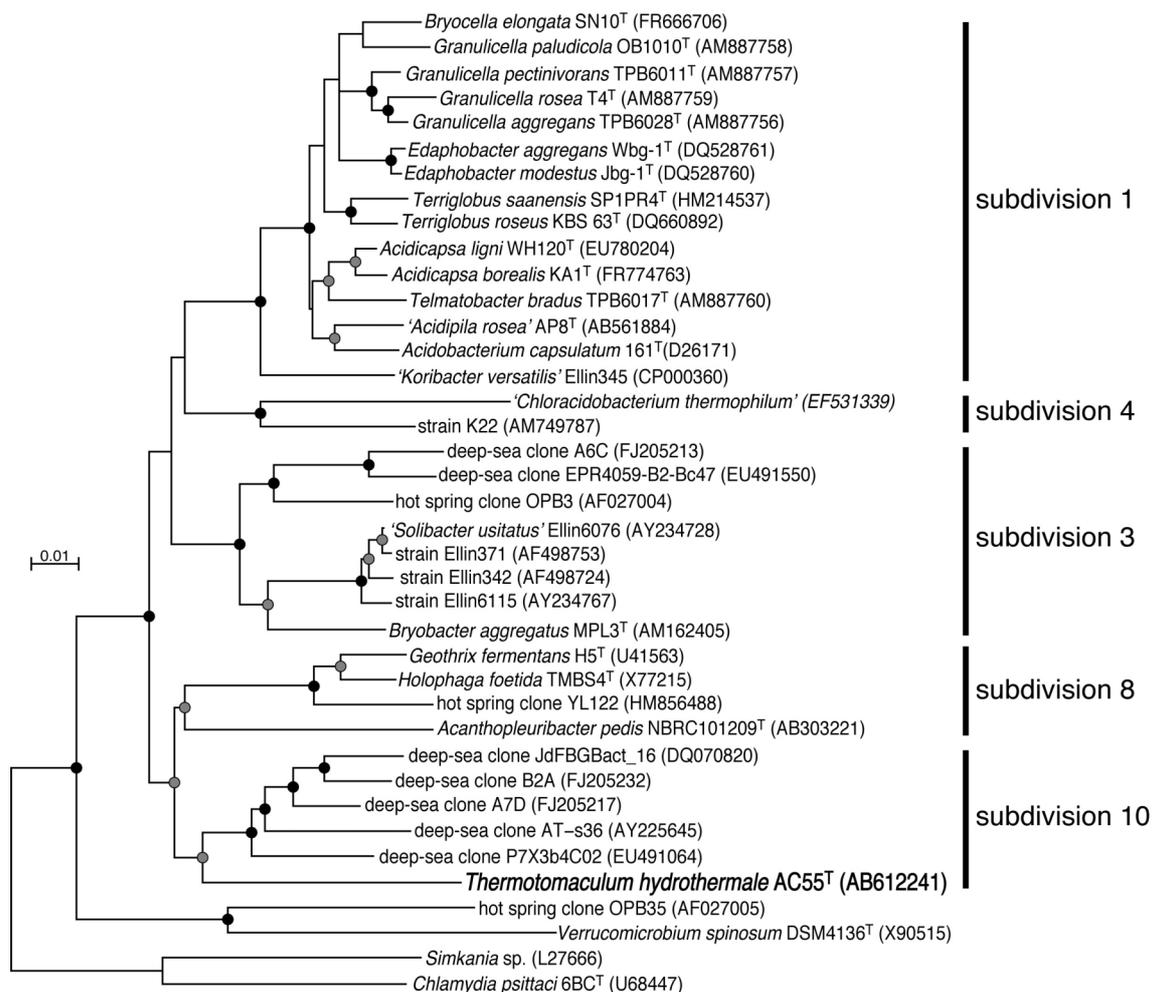


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489

490 **Figure 1.**

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Figure 2.

495 **Legend for supplementary figures.**

496 **Supplementary Fig. S1.** DAPI-stained cells of strain AC55^T in the early stationary
497 growth phase.

498

499 **Supplementary Fig. S2.** Effects of temperature (a), NaCl concentration (b) and pH (c)

500 on the growth of strain AC55^T. Growth curve at different temperatures was determined

501 in MMJYP2 medium at pH 6.0. Growth curve at different NaCl concentrations was

502 determined in the same medium at 55 °C. Growth curve at different pH was determined

503 in the same medium at 55 °C and 2.5 % (w/v) NaCl concentration.

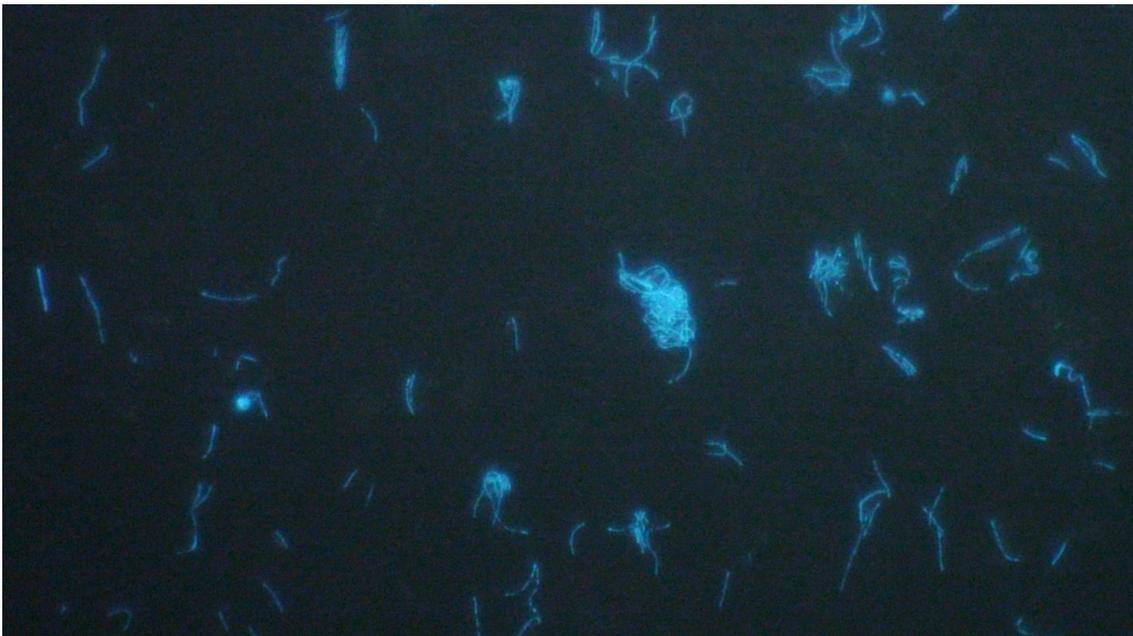
504

505 **Supplementary Fig. S3.** Polar lipid pattern of strain AC55^T. PE,

506 phosphatidylethanolamine; PL, unidentified phospholipid; APL, unidentified

507 aminophospholipid; L, unidentified lipid.

508

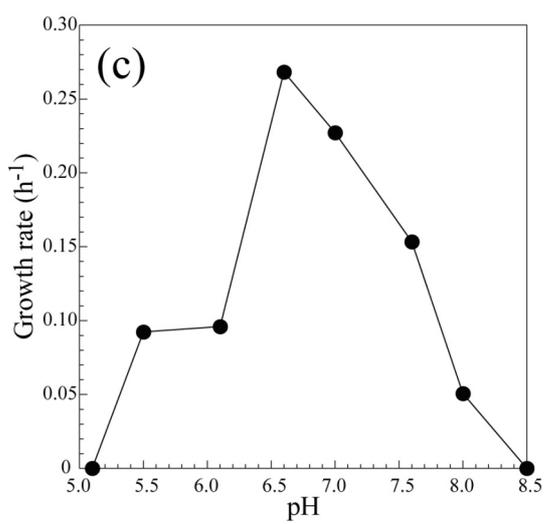
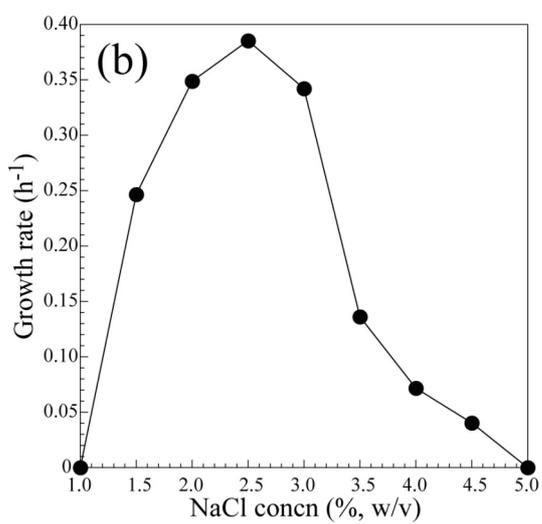
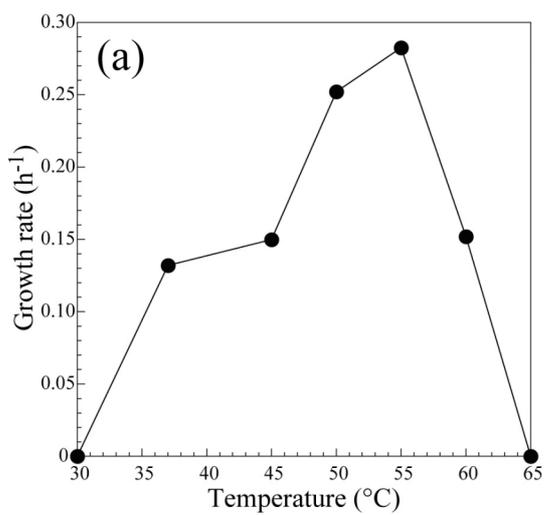


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510 Supplementary Fig. S1

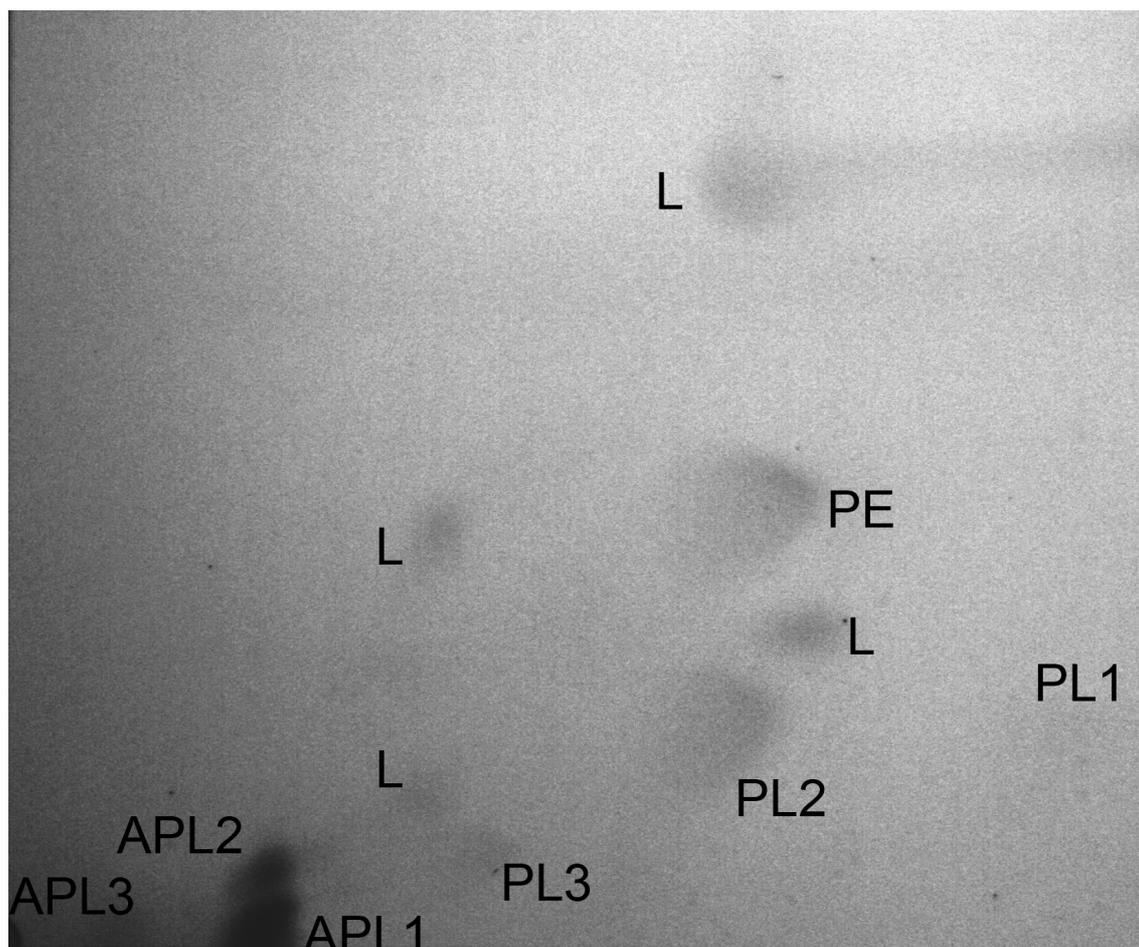
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512 Supplementary Fig. S2

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Supplementary Fig. S3