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

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Abstract

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

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

Introduction

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

dropped into a sample box. Immediately after the recovery of chimney sample onboard, a relatively large piece of structure was divided into exterior surface and vent orifice portions, and suspended in sterilized seawater in the presence of 0.05 % (w/v) neutralized sodium sulfide in a 100 ml glass bottle (Schott Glaswerke). The bottle was tightly sealed with a butyl rubber stopper under a gas phase of 100 % N_2 (200 kPa).

Cultivation The suspended slurry was used to inoculate MMJSO medium (Nunoura et al. 2007), which was further incubated at 55 °C. MMJSO medium contained 0.02 % (w/v) yeast extract, 0.05 % (w/v) pyruvate, 0.05 % (w/v) lactate, 0.1 % (w/v) $NaHCO_3$, 0.05 % (w/v) ascorbic acid, and 1 mg resazurin per liter of MJ synthetic seawater (Sako et al. 1996) under a gas mixture of H_2:CO_2 (80:20) (200kPa). MJ synthetic seawater is composed of (per liter) NaCl, 30 g; $MgCl_2 \cdot 6H_2O$, 4.18 g; $MgSO_4 \cdot 7H_2O$, 3.4 g; KCl, 0.33 g; NH_4Cl, 0.25 g; K_2HPO_4, 0.14 g; $CaCl_2 \cdot 2H_2O$, 0.14 g; and trace mineral solution, 10 ml. Trace mineral solution contains (per liter) nitrilotriacetic acid, 1.5 g; $MgSO_4 \cdot 7H_2O$, 3.0 g; $MnSO_4 \cdot 2H_2O$, 0.5 g; NaCl, 1.0 g; $FeSO_4 \cdot 7H_2O$, 0.1 g; $CoSO_4 \cdot 7H_2O$, 0.18 g; $CaCl_2 \cdot 2H_2O$, 0.1 g; $ZnSO_4 \cdot 7H_2O$, 0.18 g; $CuSO_4 \cdot 5H_2O$, 0.01 g; $KAl(SO_4)_2 \cdot 12H_2O$, 0.02 g; H_3BO_3, 0.01 g; $Na_2MoO_4 \cdot 2H_2O$, 0.01 g; $NiCl_2 \cdot 6H_2O$, 0.025 g; and $Na_2SeO_3 \cdot 5H_2O$, 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 N_2:CO_2 (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) $NaHCO_3$ and 0.05 %
106 (w/v) Na_2S in modified MJ synthetic seawater (Nakagawa and Takai 2006). Modified
107 MJ synthetic seawater is composed (per liter) of NaCl, 25 g; $MgCl_2 \cdot 6H_2O$, 4.2 g;
108 $MgSO_4 \cdot 7H_2O$, 3.4 g; KCl, 0.5 g; NH_4Cl, 0.25 g; K_2HPO_4, 0.14 g; $CaCl_2 \cdot 2H_2O$, 0.7 g.
109 To prepare MMJYP2 medium, all components other than Na_2S and $NaHCO_3$ were
110 dissolved. After autoclaving, a concentrated and filter-sterilized solution of $NaHCO_3$,
111 and neutralized Na_2S solution (pH7.5) (sterilized by autoclaving) were added to the
112 medium under gas purging of 80 % N_2 and 20 % CO_2. The tubes were then tightly
113 sealed with butyl rubber stoppers under a gas phase of 80 % N_2 + 20 % CO_2 (350 kPa).
114 No growth was observed when both $NaHCO_3$ and CO_2 were eliminated from the
115 medium.

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

Measurement of growth Growth of novel isolate was determined by direct cell counts, after staining with 6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980). To determine temperature, pH and NaCl ranges for growth, duplicate cultures were grown in 15 ml test tubes containing 3 ml medium in an incubator. Effects of pH and NaCl concentration on the growth of isolate were determined at 55 °C. NaCl requirements were determined with varying concentrations of NaCl in MMJYP2 medium from 0.5 to 5.5 % (w/v). When the pH optimum was examined, pH of the medium was readjusted immediately before inoculation with H_2SO_4 or NaOH by using a compact pH meter (Horiba AS-212) at 55 °C. The pH was found to be stable during the cultivation period.

In an attempt to examine the ability of respiratory growth, possible electron acceptors were added to MMJYP2 medium at final concentrations of 0.1 % (w/v, $Na_2S_2O_3 \cdot 5H_2O$, $NaNO_3$, ferric citrate, and Na_2SO_4), 0.1 % (v/v, O_2), 0.01-0.1 % (w/v, Na_2SO_3 and $NaNO_2$) or 1 % (w/v, S^0). O_2 was provided by injecting a defined volume

134 of O_2 (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 N_2:CO_2 (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 H_2 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 K_1 and ubiquinone-50 (coenzyme Q_{10}) 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).

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

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

Results and discussion

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

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

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

205 of fermentative growth. H_2 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) Na_2SO_3,
210 0.1 % (w/v) ferric citrate, and 0.01-0.1 % (w/v) $NaNO_2$. 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 S^0 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.

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

Description of *Thermotomaculum* gen. nov. *Thermotomaculum* (Ther.mo.to.ma'cu.lum. Gr. fem. n. *thermê*, heat; L. neut. n. *tomaculum*, a kind of sausage; N.L. neut. n. *Thermotomaculum*, a sausage-shaped thermophile). Non-motile rods that stain Gram-negative. Anaerobic. Thermophilic. Heterotrophic. Growth by fermentation. Major cellular fatty acids are $C_{17:0}$ and $C_{15:0}$. Major quinones are menaquinone-7 and -8. Major polar lipids are phosphatidylethanolamine, unidentified aminophospholipids, and unidentified phospholipids. Members of the genus *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 °C (optimum
263 55 °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.

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272

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Table 1. Comparison of major characteristics of strain AC55^T with those of other members of the phylum *Acidobacteria*.
Strains: 1, *Thermotomaculum hydrothermale* AC55^T; 2, *Acidobacterium capsulatum* 161^T (Kishimoto et al. 1991); 3, *Edaphobacter* spp. (Koch et al. 2008); 4, *Acidicapsa* spp. (Kulichevskaya et al. 2011); 5, *Granulicella* spp. (Pankratov and Dedysh 2010); 6, *Terriglobus* spp. (Eichorst et al. 2007; Männistö et al. 2010); 7, *Bryocella elongata* SN10^T (Dedysh et al. 2011); 8, *Telmatobacter bradus* TPB6017^T (Pankratov et al. 2011); 9, *Bryobacter aggregatus* MPL3^T (Kulichevskaya et al. 2010); 10, *Acanthopleuribacter pedis* FYK2218^T (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 *Cladonia*	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

ND, not determined

*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.

486

487

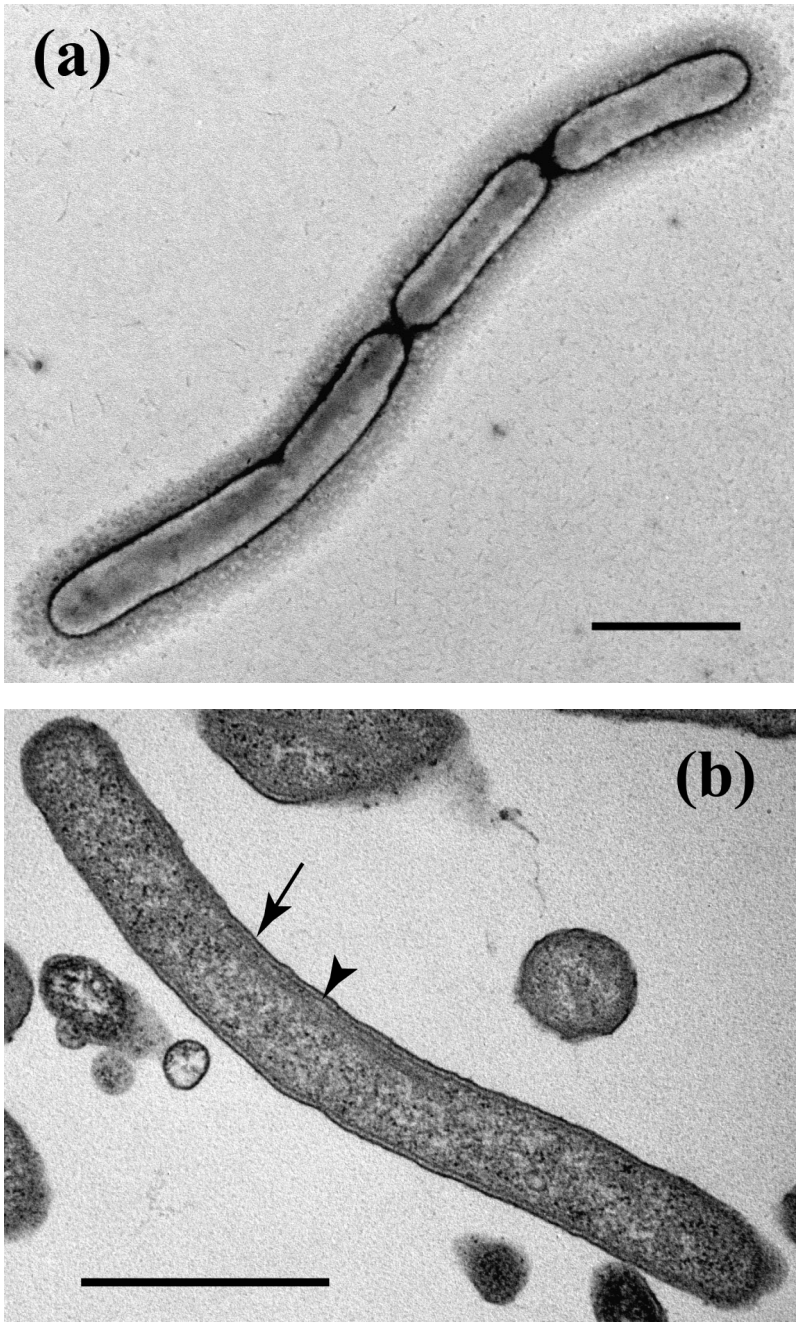
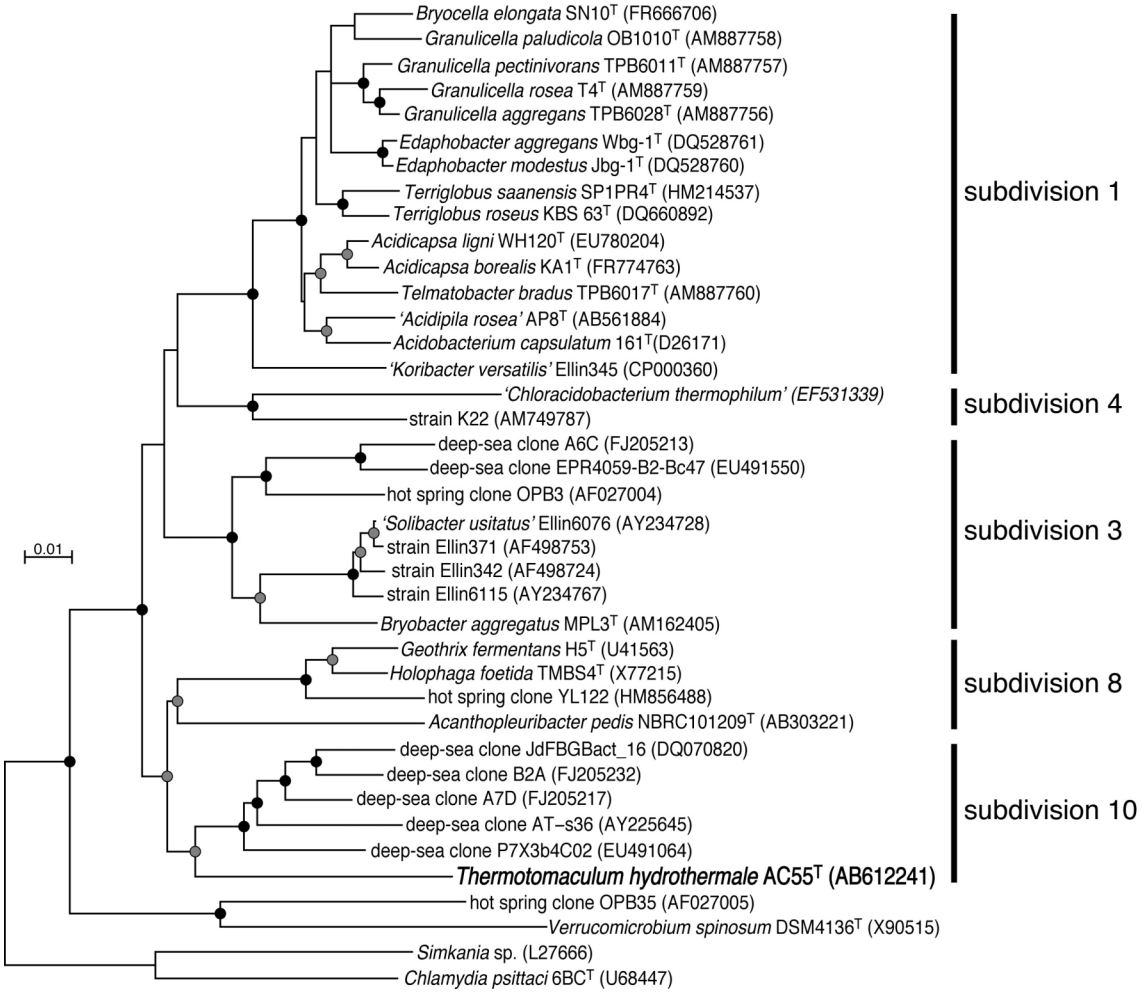


Figure 1.


Bryocella elongata SN10T (FR666706)
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Granulicella pectinivorans TPB6011T (AM887757)
Granulicella rosea T4T (AM887759)
Granulicella aggregans TPB6028T (AM887756)
Edaphobacter aggregans Wbg-1T (DQ528761)
Edaphobacter modestus Jbg-1T (DQ528760)
Terriglobus saanensis SP1PR4T (HM214537)
Terriglobus roseus KBS 63T (DQ660892)
Acidicapsa ligni WH120T (EU780204)
Acidicapsa borealis KA1T (FR774763)
Telmatobacter bradus TPB6017T (AM887760)
'Acidipila rosea' AP8T (AB561884)
Acidobacterium capsulatum 161T(D26171)
'Koribacter versatilis' Ellin345 (CP000360)
'Chloracidobacterium thermophilum' (EF531339)
strain K22 (AM749787)
deep-sea clone A6C (FJ205213)
deep-sea clone EPR4059-B2-Bc47 (EU491550)
hot spring clone OPB3 (AF027004)
'Solibacter usitatus' Ellin6076 (AY234728)
strain Ellin371 (AF498753)
strain Ellin342 (AF498724)
strain Ellin6115 (AY234767)
Bryobacter aggregatus MPL3T (AM162405)
Geothrix fermentans H5T (U41563)
Holophaga foetida TMBS4T (X77215)
hot spring clone YL122 (HM856488)
Acanthopleuribacter pedis NBRC101209T (AB303221)
deep-sea clone JdFBGBact_16 (DQ070820)
deep-sea clone B2A (FJ205232)
deep-sea clone A7D (FJ205217)
deep-sea clone AT–s36 (AY225645)
deep-sea clone P7X3b4C02 (EU491064)
Thermotomaculum hydrothermale AC55T (AB612241)
hot spring clone OPB35 (AF027005)
Verrucomicrobium spinosum DSM4136T (X90515)
Simkania sp. (L27666)
Chlamydia psittaci 6BCT (U68447)
0.01
subdivision 1
subdivision 4
subdivision 3
subdivision 8
subdivision 10

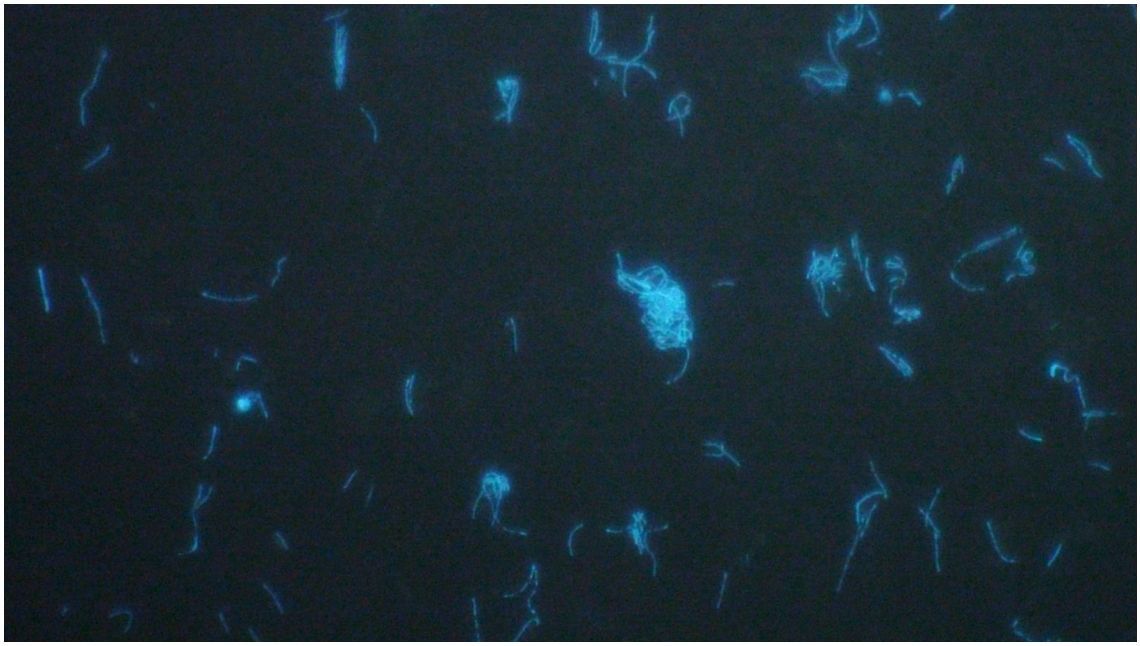
Figure 2.

Legend for supplementary figures.

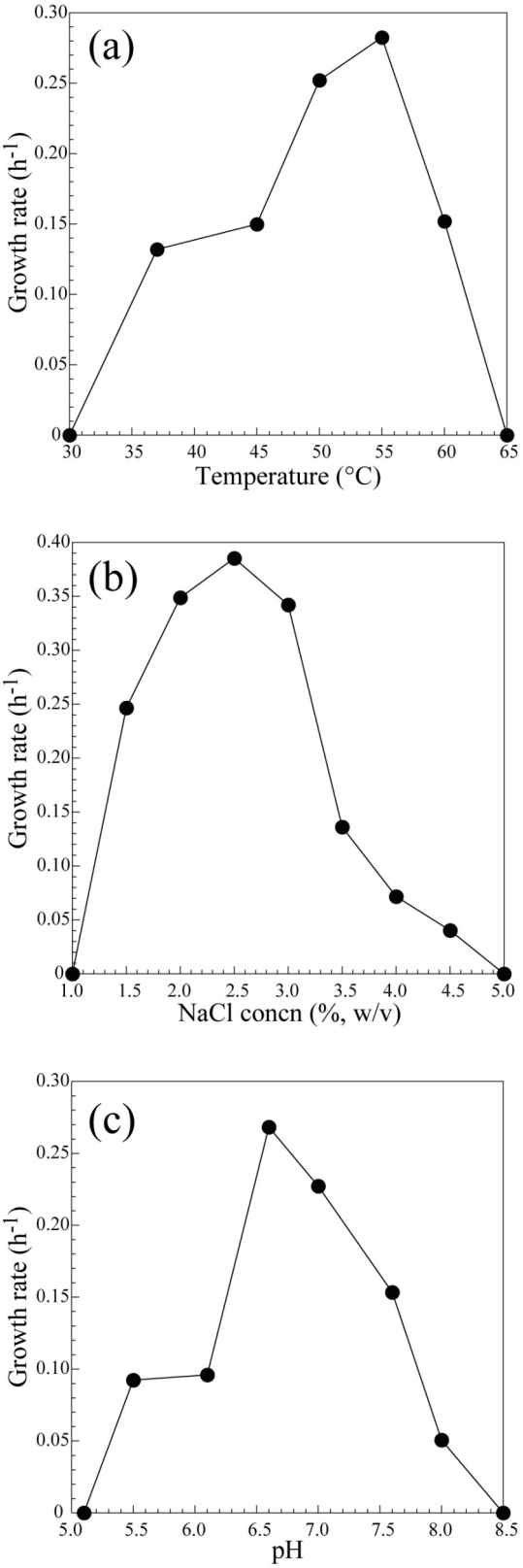
Supplementary Fig. S1. DAPI-stained cells of strain AC55^T in the early stationary growth phase.

Supplementary Fig. S2. Effects of temperature (a), NaCl concentration (b) and pH (c) on the growth of strain AC55^T. Growth curve at different temperatures was determined in MMJYP2 medium at pH 6.0. Growth curve at different NaCl concentrations was detrermined in the same medium at 55 °C. Growth curve at different pH was determined in the same medium at 55 °C and 2.5 % (w/v) NaCl concentration.

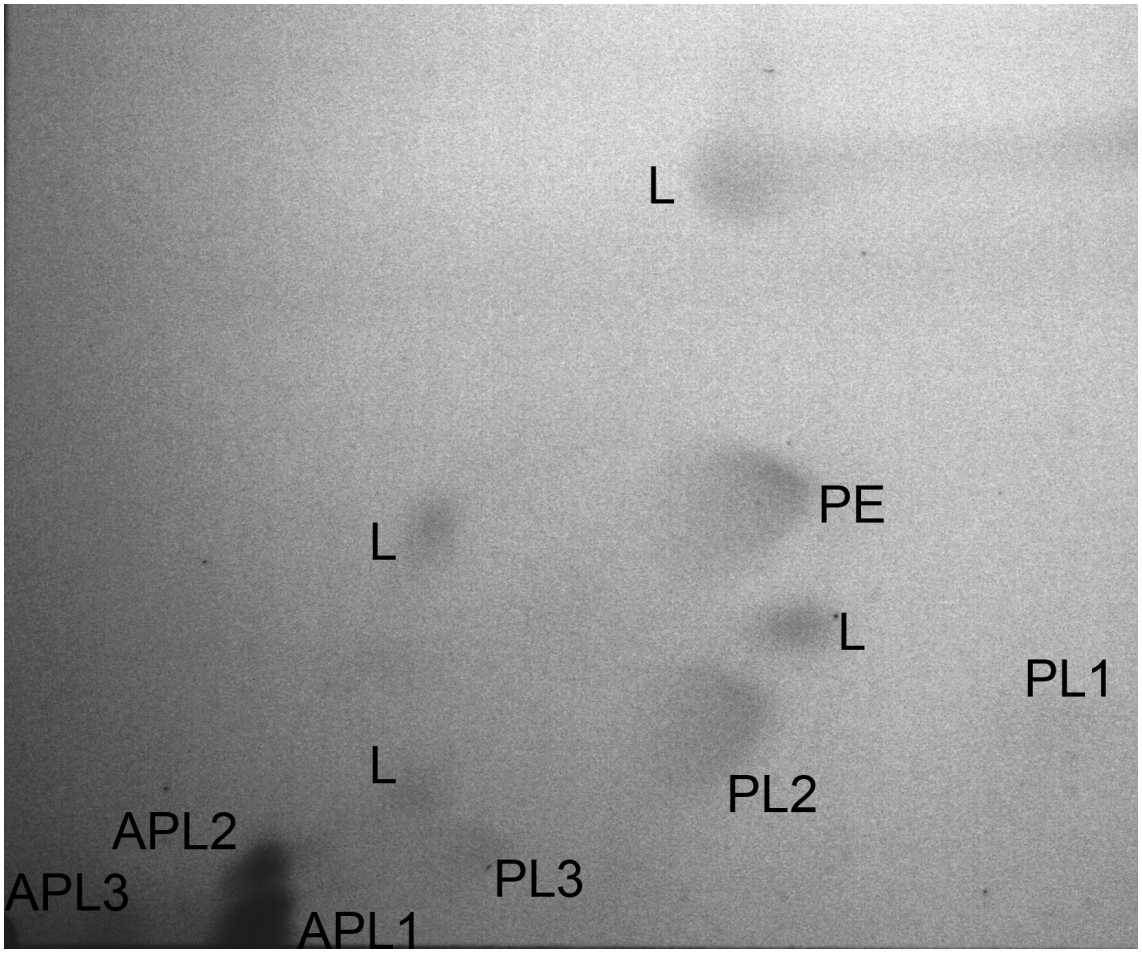
Supplementary Fig. S3. Polar lipid pattern of strain AC55^T. PE, phosphatidylethanolamine; PL, unidentified phospholipid; APL, unidentified aminophospholipid; L, unidentified lipid.



Supplementary Fig. S1



Supplementary Fig. S2



Supplementary Fig. S3