

# ***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<sup>T</sup>, was isolated from a deep-sea hydrothermal vent chimney at the Hatoma Knoll in the Okinawa Trough, Japan. Cells of strain AC55<sup>T</sup> were non-motile, long rods (2.0- to 6.8-μm long and 0.3- to 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<sup>T</sup> was 51.6 mol%. The 16S rRNA gene sequence analysis revealed that strain AC55<sup>T</sup> 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<sup>T</sup> (=JCM 17643<sup>T</sup> = DSM 24660<sup>T</sup> = NBRC 107904<sup>T</sup>).

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

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## **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<sup>T</sup> (accession no. NC\_012483), *Terrioglobus saanensis* SP1PR4<sup>T</sup> (accession no. NC\_014963), *Granulicella tundricola* MP5ACTX9<sup>T</sup> (accession no. NC\_015064), ‘*Koribacter versatilis*’ strain Ellin345 (subdivision 1; NC\_008009), ‘*Solibacter usitatus*’ strain Ellin6076 (subdivision 3; NC\_008536) (Ward et al. 2009) and ‘*Candidatus Chloracidobacterium thermophilum*’ (Garcia Costas et al. 2011).

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

## Materials and methods

### Sample collection

A sample from a deep-sea hydrothermal vent chimney was obtained from the Hatoma Knoll (24°51′N, 123°50′E) in the Southern Okinawa Trough at a depth of 1470 m by means of a ROV Hyper Dolphin in July 2008. The chimney 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<sub>2</sub> (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<sub>3</sub>, 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<sub>2</sub>:CO<sub>2</sub> (80:20) (200 kPa). MJ synthetic seawater is composed of (per liter) NaCl, 30 g; MgCl<sub>2</sub>·6H<sub>2</sub>O, 4.18 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 3.4 g; KCl, 0.33 g; NH<sub>4</sub>Cl, 0.25 g; K<sub>2</sub>HPO<sub>4</sub>, 0.14 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.14 g; and trace mineral solution, 10 ml. Trace mineral solution contains (per liter) nitrilotriacetic acid, 1.5 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 3.0 g; MnSO<sub>4</sub>·2H<sub>2</sub>O, 0.5 g; NaCl, 1.0 g; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g; CoSO<sub>4</sub>·7H<sub>2</sub>O, 0.18 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.1 g; ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.18 g; CuSO<sub>4</sub>·5H<sub>2</sub>O, 0.01 g; KAl(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O, 0.02 g; H<sub>3</sub>BO<sub>3</sub>, 0.01 g; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, 0.01 g; NiCl<sub>2</sub>·6H<sub>2</sub>O, 0.025 g; and Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O, 0.3 mg.

The presence or absence of cell growth was determined by microscopic observation. In order to obtain consistent growth, gas phase of the MMJSO medium was changed to a gas mixture of N<sub>2</sub>:CO<sub>2</sub> (80:20) (200 kPa). To obtain a pure culture, a dilution-to-extinction method was employed at 55°C and repeated at least five times (Baross 1995). Purity was confirmed routinely by microscopic observation and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

The isolate was routinely cultivated in MMJYP2 medium, which contains 0.4% (w/v) yeast extract, 0.4% (w/v) tryptone peptone, 0.1% (w/v) NaHCO<sub>3</sub> and 0.05% (w/v) Na<sub>2</sub>S in modified MJ synthetic seawater (Nakagawa and Takai 2006). Modified MJ synthetic seawater is composed (per liter) of NaCl, 25 g; MgCl<sub>2</sub>·6H<sub>2</sub>O, 4.2 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 3.4 g; KCl, 0.5 g; NH<sub>4</sub>Cl, 0.25 g; K<sub>2</sub>HPO<sub>4</sub>, 0.14 g; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.7 g. To prepare MMJYP2 medium, all components other than Na<sub>2</sub>S and NaHCO<sub>3</sub> were dissolved. After autoclaving, a concentrated and filter-sterilized solution of NaHCO<sub>3</sub>, and neutralized Na<sub>2</sub>S solution (pH 7.5) (sterilized by autoclaving) were added to the medium under gas purging of 80% N<sub>2</sub> and 20% CO<sub>2</sub>. The tubes were then tightly sealed with butyl rubber stoppers under a gas phase of 80% N<sub>2</sub> + 20% CO<sub>2</sub> (350 kPa). No growth was observed when both NaHCO<sub>3</sub> and CO<sub>2</sub> were eliminated from the medium.

### Light and electron microscopy

Cells were routinely observed using a ZEISS Axiophot microscope (Carl Zeiss). Transmission electron micrographs of negatively stained 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<sub>2</sub>SO<sub>4</sub> or NaOH 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<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O, NaNO<sub>3</sub>, ferric citrate, and Na<sub>2</sub>SO<sub>4</sub>), 0.1% (v/v, O<sub>2</sub>), 0.01–0.1% (w/v, Na<sub>2</sub>SO<sub>3</sub> and NaNO<sub>2</sub>) or 1% (w/v, S<sup>0</sup>). O<sub>2</sub> was provided by injecting a defined volume of O<sub>2</sub> (0.1–10%, v/v) into the culture tubes as previously described (Nakagawa et al. 2003). The production of hydrogen sulfide was detected using lead acetate solution.

In an attempt to find organic substrates that could support the growth of isolate, experiments were conducted in which the yeast extract and tryptone peptone in MMJYP2 medium were replaced with other organic materials as potential substrates under a gas phase of N<sub>2</sub>:CO<sub>2</sub> (80:20, 350 kPa). Each of the following substrates was added at concentrations of 0.01% or 0.1% (w/v): L-cystine, L-phenylalanine, L-proline, Casamino acids, (+)-D-glucose, lactose, maltose, chitin, starch, cellulose, formate, formaldehyde, acetate, citrate, pyruvate, propionate, methanol, tryptone peptone and yeast extract (Difco). Products of fermentative growth were identified with F-kit (Roche Applied Science, USA) and H<sub>2</sub> detector tube (Gastec, Japan). Chemolithoautotrophic growth was examined as described in Nakagawa et al. (2005).

### Lipid components

Respiratory lipoquinones and polar lipids were extracted from freeze-dried cells following Minnikin et al. (1984). Cells grown in MMJYP2 medium at 55°C in the late-exponential phase of growth were used. Respiratory lipoquinones were dissolved in petroleum ether and applied to TLC plates (silica gel). After development with hexane–benzene–chloroform (5:2:1, v/v) separated components were detected at UV-254 nm. Standards of vitamin K<sub>1</sub> and ubiquinone-50 (coenzyme Q<sub>10</sub>) were used to locate bands corresponding to menaquinone and ubiquinone, respectively. UV-absorbing bands were removed from the plates and further analyzed using a Shimadzu HPLC with a reverse phase Kinetex C18 column and methanol–isopropanol (3:1, v/v) as the mobile phase at 1 ml/min at 37°C and were detected at 269 nm (Tamaoka et al. 1983). Polar lipids were separated by two-dimensional silica gel TLC as described in Pankratov et al. (2011). The plates were

sprayed with molybdophosphoric acid (total lipids), molybdenum blue (phospholipids), ninhydrin (free amino groups) and  $\alpha$ -naphthol reagents (glycolipids) and Dragendorff reagent (quaternary nitrogen). The standards of phospholipids (Sigma, USA) were used for diagram disposition of phospholipids during comparative analysis.

For fatty acid analysis, lyophilized cells were placed in a Teflon-lined, screw-capped tube containing 1 ml of anhydrous methanolic HCl and heated at 100°C for 3 h. The extraction and analysis of fatty acid methyl esters have been described previously (Komagata and Suzuki 1987). For comparative purposes, type strains of *Acidobacterium capsulatum* (JCM7670), *Staphylococcus epidermidis* (JCM2414), *Streptomyces olivaceus* (JCM4066), and *Leifsonia shinshuensis* (JCM10591) were used.

### DNA base composition

Genomic DNA was isolated by a standard 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).

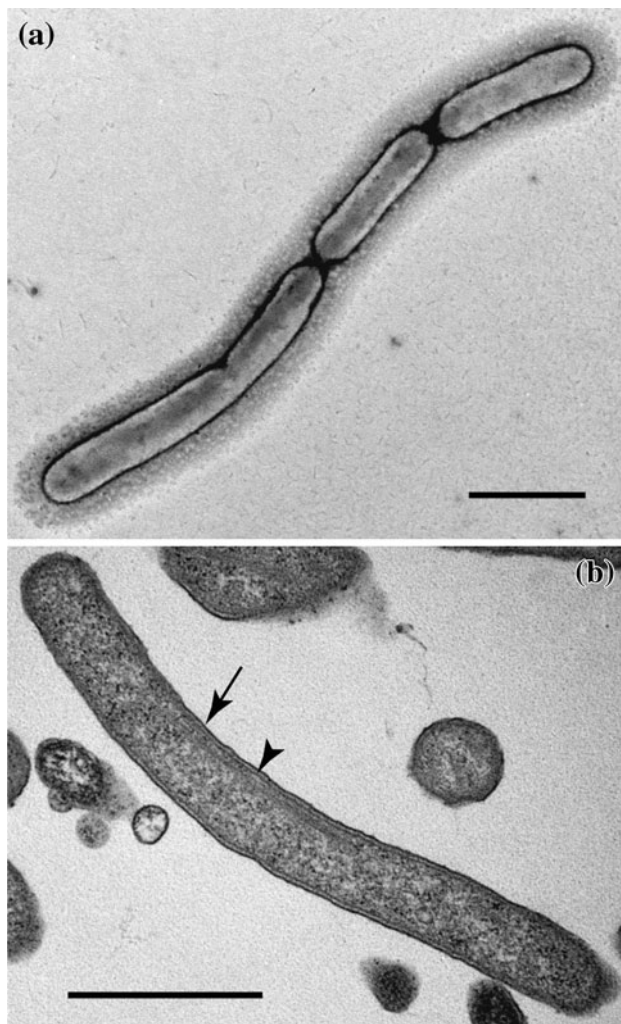
### 16S rRNA gene analysis

The 16S rRNA gene was amplified by PCR using primers Eubac 27F and 1492R (Lane 1991). Sequence of the PCR product (1412 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. 1990). 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 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<sup>T</sup> and investigated in



**Fig. 1** Electron micrograph of a negatively stained cell (a) and thin section (b) of strain AC55<sup>T</sup>. Arrowhead cytoplasmic membrane, arrow outer membrane. Bars 2.0 μm (a) and 1.0 μm (b)

detail. Cells of strain AC55<sup>T</sup> 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  $4.0 \times 10^7$  cells/ml, respectively. No growth was observed at 30 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 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 of fermentative growth. H<sub>2</sub> formation was not detected (detection limit  $\geq 0.5\%$ , v/v). L-cystine, L-phenylalanine, L-proline, Casamino acids, (+)-D-glucose, lactose, maltose, chitin, starch, cellulose, formate, formaldehyde, acetate, citrate, pyruvate, propionate, methanol, 0.01% (w/v) tryptone peptone and yeast extract did not support the growth.

The growth of strain AC55<sup>T</sup> was inhibited by the addition of 0.1% (w/v) Na<sub>2</sub>SO<sub>3</sub>, 0.1% (w/v) ferric citrate, and 0.01–0.1% (w/v) NaNO<sub>2</sub>. In other cases, possible electron acceptors used in this study resulted in no significant differences in growth rate or in maximal yield, although S<sup>0</sup> was reduced to hydrogen sulfide.

#### Lipid components

Strain AC55<sup>T</sup> contained menaquinone-8 (MK-8; 85.6%) and -7 (MK-7; 14.4%) as the predominant isoprenoid quinones. Members of the phylum *Acidobacteria* subdivision 1 also contained MK-8 as the predominant isoprenoid quinones but not MK-7 (Table 1). As shown by TLC, strain AC55<sup>T</sup> possesses phosphatidylethanolamine, unidentified aminophospholipids, and unidentified phospholipids (Supplementary Fig. S3). The cellular fatty acids of strain AC55<sup>T</sup> were C<sub>17:0</sub> (66.7%), C<sub>15:0</sub> (26.2%), C<sub>14:0</sub>.OH (4.6%), and C<sub>16:0</sub> (2.5%). The dominance of odd-chain fatty acids is a shared feature among acidobacteria.

#### DNA base composition

The G + C content of genomic DNA from strain AC55<sup>T</sup> was 51.6 mol% (Table 1).

#### Phylogenetic analysis

The 16S rRNA gene sequence of strain AC55<sup>T</sup> was applied to sequence similarity analysis with databases by the BLAST search algorithm (Altschul et al. 1990). Among the species with validly published names, *Holophaga foetida* (85%) and *Geothrix fermentans* (84%) were the closest relatives of the isolate. The phylogenetic tree indicated that strain AC55<sup>T</sup> was the first cultivated member of subdivision 10 within the phylum *Acidobacteria* (Fig. 2). This



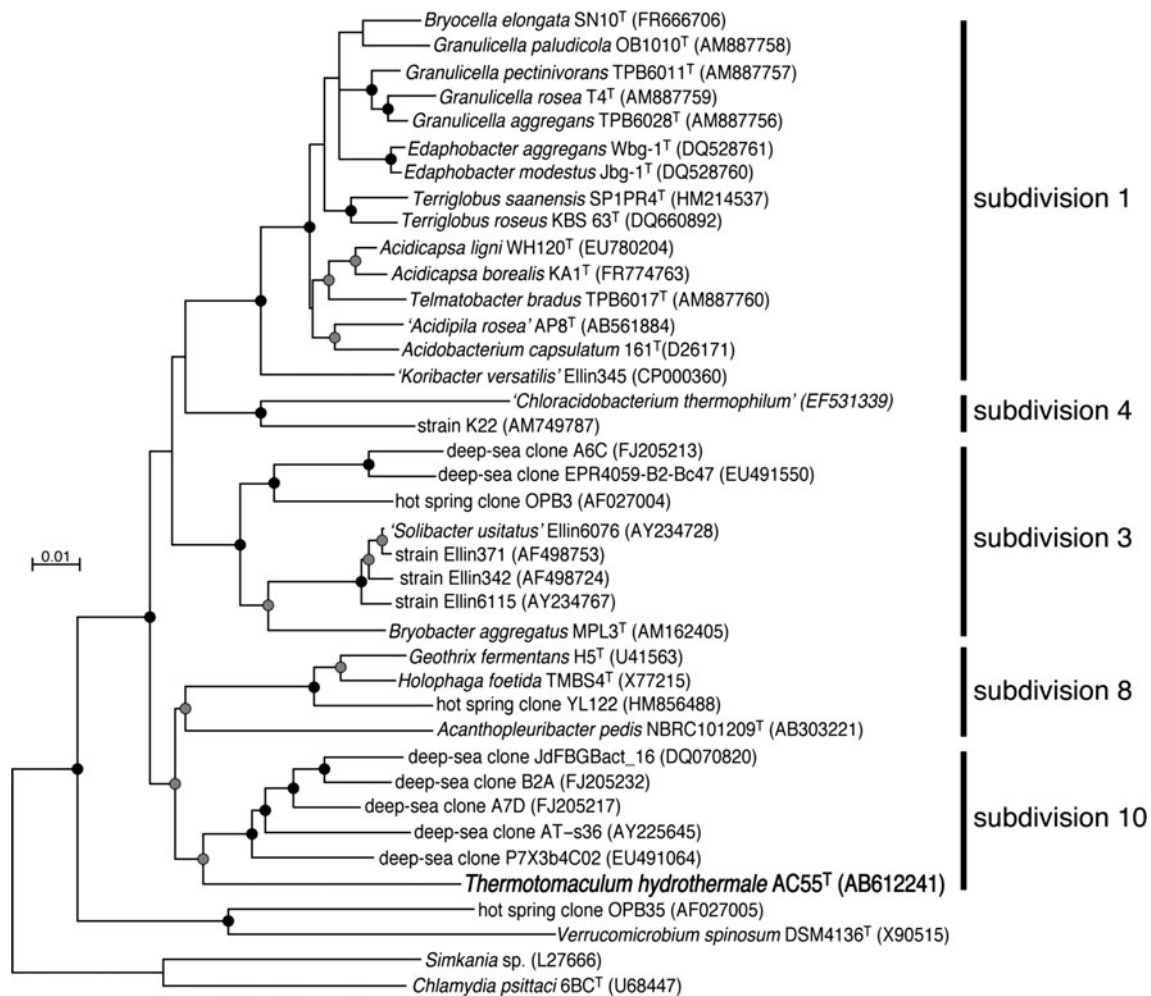
**Table 1** Comparison of major characteristics of strain AC55<sup>T</sup> with those of other members of the phylum *Acidobacteria*

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 forest soil	Peat and wood	Peat and <i>Cladonia</i>	Soil and termitite 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 <sup>a</sup>	ND	ND
NaCl optimum (%)	2.5	ND	ND	ND	ND	ND	ND	ND	ND	ND <sup>a</sup>	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

Strains: 1, *Thermotomaculum hydrothermale* AC55<sup>T</sup>; 2, *Acidobacterium capsulatum* 161<sup>T</sup> (Kishimoto et al. 1991); 3, *Edaphobacter* spp. (Koch et al. 2008); 4, *Acidicapsa* spp. (Kulichkevskaya 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<sup>T</sup> (Dedysh et al. 2011); 8, *Telmatobacter bradus* TPB6017<sup>T</sup> (Pankratov et al. 2011); 9, *Bryobacter aggregatus* MPL3<sup>T</sup> (Kulichkevskaya et al. 2010); 10, *Acanthopleuribacter pedis* FYK2218<sup>T</sup> (Fukunaga et al. 2008); 11, *Geothrix fermentans* H-5<sup>T</sup> (Coates et al. 1999); 12, *Holophaga foetida* TMBS4<sup>T</sup> (Liesack et al. 1994)

ND not determined

<sup>a</sup> Growth was observed on R2A agar containing 50–150% artificial seawater (optimum 70–120% ASW)



**Fig. 2** Neighbor-joining phylogenetic tree based on 990 aligned positions of the 16S rRNA gene sequence. Bootstrap analyses (100 replications for the maximum-likelihood and 1000 replications for the neighbor-joining) were used to obtain confidence estimates for the tree topology. Branch points conserved with bootstrap values of

>75% (solid circles) and with bootstrap values of >50% (gray circles) with the both neighbour-joining and maximum-likelihood methods are indicated. The accession numbers for sequences are given in parentheses. The scale bar represents the expected number of changes per nucleotide position

subdivision contained environmental clone sequences retrieved from various deep-sea habitats, including hydrothermal sediments (López-García et al. 2003) and basaltic lavas (Santelli et al. 2008) (Fig. 2).

#### Comparison with related genera

A number of fermentative thermophiles and hyperthermophiles, such as members of the *Thermococcales* and *Thermotogales*, have been found in deep-sea hydrothermal environments (Takai et al. 2006; Nakagawa and Takai 2008). Recently, additional lineages of deep-sea thermophilic fermenters have been characterized (Reysenbach et al. 2006; Imachi et al. 2008), suggesting the diversity of fermenters in deep-sea vents might still be underestimated. Although differences in their growth strategies in situ

remain to be studied, strain AC55<sup>T</sup> is unique in that its growth is not stimulated by elemental sulfur.

Strain AC55<sup>T</sup> 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 that 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 also 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<sup>T</sup> (=JCM 17643<sup>T</sup> = DSM 24660<sup>T</sup> = NBRC 107904<sup>T</sup>).

#### Description of *Thermotomaculum* gen. nov.

*Thermotomaculum* (Ther.mo.to.ma'cu.lum. Gr. fem. n. *thermé*, 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<sub>17:0</sub> and C<sub>15:0</sub>. 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 *Thermotomaculum hydrothermale*.

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

*Thermotomaculum hydrothermale* (hy.dro.ther.ma'le. N.L. neut. adj. *hydrothermale*, pertaining to a hydrothermal vent). Cells are non-motile, with a mean length of 2.0–6.8 µm and width of approximately 0.3–0.6 µm. The temperature range for growth is 37–60°C (optimum 55°C). The pH range for growth is 5.5–8.5 (optimum 6.6). NaCl in the concentration range for growth is 15–45 g/l (optimum 25 g/l). Fermentative growth occurs with yeast extract, tryptone peptone as the sole carbon and energy source. The major cellular fatty acids are C<sub>17:0</sub> and C<sub>15:0</sub>. The G + C content of the genomic DNA is 51.6 mol%, isolated from a deep-sea hydrothermal vent in the Southern Okinawa Trough, Japan. The type strain is AC55<sup>T</sup> (=JCM 17643<sup>T</sup> = DSM 24660<sup>T</sup> = NBRC 107904<sup>T</sup>). The DDBJ/EMBL/GenBank accession number for the 16S rRNA gene of strain AC55<sup>T</sup> is AB612241.

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