Aciditerrimonas ferrireducens gen. nov., sp. nov., an iron-reducing thermoacidophilic actinobacterium isolated from a solfataric field

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An iron-reducing, moderately thermophilic, acidophilic actinobacterium, strain IC-180^T, isolated from a solfataric field in Hakone, Japan, was subjected to polyphasic taxonomic analysis. Strain IC-180^T was a motile, short rod-shaped, Gram-positive bacterium that was able to grow at temperatures of 35–58 °C (optimally at 50 °C) and at pH 2.0–4.5 (optimally at pH 3.0). The strain grew aerobically and heterotrophically. It also grew anaerobically or autotrophically by dissimilatory reduction of ferric iron. No oxidation of ferrous iron was observed. Major cellular fatty acids detected were iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{18:0}; the major menaquinone was MK-9(H₈). Phosphatidyl-*N*-methylethanolamine and an unknown ninhydrin-positive phosphoglycolipid were detected. The total DNA G+C content was 74.1 mol%. 16S rRNA gene sequence comparisons revealed that strain IC-180^T was a member of the order *Acidimicrobiales* and clustered coherently with uncultured actinobacteria from a geothermal site and a bioreactor operated under moderately thermophilic conditions. This cluster could be distinguished from the two other clusters comprising the families of this order, *Acidimicrobiaceae* and *Iamiaceae*, respectively. Based on the properties of strain IC-180^T determined in this polyphasic taxonomic study, this strain represents a novel species in a new genus in the order *Acidimicrobiales*, for which the name *Aciditerrimonas ferrireducens* gen. nov., sp. nov. is proposed; the type strain is IC-180^T (=JCM 15389^T =DSM 45281^T).

Solfataric fields, or geothermally heated areas associated with fumaroles emitting sulfurous gases containing H₂S and SO₂, are located in various terrestrial regions near tectonically active zones. In such environments, extreme acidity, which is attributed to abiotic and biotic processes, including oxidation of H₂S to sulfur with atmospheric oxygen followed by oxidation of sulfur to sulfuric acid by sulfur-oxidizing bacteria and archaea, and elevated temperatures allow minerals to be partially or completely broken down (Brock, 1986; Johnson, 2007). Thus, solfataric fields harbour a variety of thermoacidophilic bacteria and archaea mediating geochemical processes. In an attempt to isolate various thermoacidophilic microorganisms from a solfataric field in Hakone, Japan (Itoh *et al.*, 2007), a novel thermoacidophilic bacterial strain, designated IC-180^T, which was phylogenetically affiliated

with acidophilic actinobacteria in the order *Acidimicrobiales* (Stackebrandt *et al.*, 1997; Zhi *et al.*, 2009), was isolated. At the time of writing, the order *Acidimicrobiales* comprises two families, *Acidimicrobiaceae* and *Iamiaceae* (Zhi *et al.*, 2009; Kurahashi *et al.*, 2009). The former family comprises three monospecific genera, *Acidimicrobium* (Clark & Norris, 1996), *Ferrimicrobium* and *Ferrithrix* (Johnson *et al.*, 2009), members of which are found in mineral-rich acidic environments such as mines and solfataric fields. In addition, a recently described genus with a name that has not yet been validly published, '*Acidithiomicrobium*', is also affiliated with this family (Davis-Belmar & Norris, 2009). In contrast, the latter family comprises the non-acidophilic species *Iamia majanohamensis*, which is associated with a marine animal (Kurahashi *et al.*, 2009). In addition, the actinobacterium *Ilumatobacter fluminis*, isolated from estuary sediment (Matsumoto *et al.*, 2009), may be included in the order *Acidimicrobiales*. The three genera of the family *Acidimicrobiaceae* are known to be mesophilic or moderately

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of IC-180^T is AB517669.

A supplementary figure is available with the online version of this paper.

thermophilic, extremely acidophilic, and capable of oxidation and reduction of iron (Clark & Norris, 1996; Bridge & Johnson, 1998; Johnson *et al.*, 2009). In this paper, the morphological, physiological, biochemical, chemotaxonomic and phylogenetic properties of strain IC-180^T were determined to reveal its taxonomic position. It is proposed that this isolate represents a novel species in a new genus in the order *Acidimicrobiales*.

Strain IC-180^T was isolated from a solfataric soil sample (60 °C, pH 1.4) collected at a solfataric field, Ohwaku-dani, Hakone, Japan, by applying the most-probable number method using *Sulfolobus* medium with 1.0 g yeast extract l⁻¹ (Brock *et al.*, 1972) at pH 2.5 and 55 °C, as described previously (Itoh *et al.*, 2007). It was the sole bacterial isolate from the sample, although several thermoacidophilic archaeal strains, which were identified or provisionally assigned by 16S rRNA gene comparisons to the species *Thermogymnomonas acidicola*, *Acidianus infernus*, *Acidianus brierleyi*, *Metallosphaera hakonensis* and *Picrophilus oshimae*, were isolated from the same sample. The purity of the bacterial strain was confirmed by microscopic observation of cell morphology and repeated partial sequencing of the 16S rRNA gene using several primers. Strain IC-180^T was routinely cultivated aerobically at 50 °C in modified *Sulfolobus* medium (the medium was amended with 1.0 g yeast extract l⁻¹, but FeCl₃·6H₂O was omitted, pH 2.5). Unless otherwise indicated, this medium was used as the basal medium for physiological and biochemical experiments. *Acidimicrobium ferrooxidans* JCM 15462^T was used as a reference strain.

Cells of strain IC-180^T were Gram-positive, short rods (0.5–0.6 × 0.8–1.1 µm), occurring singly, in pairs or short chains during exponential to early stationary growth phase. They were motile, exhibiting tumbling motion. No endospores were observed. The platinum-palladium shadowed cells revealed peritrichous flagellation under a transmission electron microscope (as shown in Fig. 1).

Fig. 1. Transmission electron micrograph of a cell with peritrichous flagella shadowed with platinum-palladium. Bar, 1 µm.

Colonies formed on an agar (2 %, w/v) plate of modified *Sulfolobus* medium incubated aerobically for 1 week were tiny (less than 0.2 mm in diameter), convex and white to cream in colour.

Under air, strain IC-180^T grew between 35 and 58 °C and at pH 2.0–4.5, with optimal growth at 50 °C and pH 3.0; no growth was observed at 30 or 60 °C or at pH 1.5 or 5.0. The isolate grew heterotrophically using yeast extract. Alternatively, it utilized glucose, lactose, maltose, sucrose, xylose and glycogen as sole carbon and energy sources (at 0.5 %, w/v). Fructose, galactose, mannose, ribose and starch supported weak growth, whereas arabinose, acetate, butyrate, citrate, formate, fumarate, lactate, propionate and succinate did not (carbohydrates at 0.5 %; organic acids at 0.2 %). Addition of ferrous sulfate (10 mM) or synthesized iron disulfide (FeS₂, 80 mM) in the modified *Sulfolobus* medium did not affect growth and these substances were not oxidized.

Under anaerobic conditions (gas phase, N₂), growth was observed in the modified *Sulfolobus* medium supplemented with 10 mM ferric sulfate. The modified *Sulfolobus* medium without ferric sulfate did not support anaerobic growth, even if supplemented with sulfate, nitrate (10 mM each) or sulfur (30 mM). Anaerobic growth in the presence of 10 mM ferric sulfate was accompanied by the reduction of ferric iron [as indicated by the increase of ferrous iron according to the ferrozine assay as described by Phillips & Lovley (1987); see Supplementary Fig. S1 available in IJSEM Online]. Ferric sulfate could be replaced with poorly crystalline Fe(III) oxide (Lovley & Phillips, 1986), ferric chloride, ferric citrate or Mn(IV) oxide, but not with Fe(III). EDTA (all at 10 mM). On the other hand, yeast extract could be replaced with glucose, lactose, maltose, mannose, xylose, glycogen, citrate and pyruvate, but not with arabinose, fructose, galactose, ribose, sucrose, starch, acetate, butyrate, formate, fumarate, lactate, malate, propionate or succinate (carbohydrates at 0.5 %, w/v; organic acids at 0.2 %, w/v). In the presence of ferric iron, autotrophic growth occurred under a gas mixture of H₂/CO₂ (4:1, v/v), but not in N₂/CO₂ (4:1, v/v), indicating that H₂ acted as an electron donor.

Cells of strain IC-180^T contained *meso*-diaminopimelic acid as a diagnostic cell wall amino acid, according to the method of Hasegawa *et al.* (1983). The cellular fatty acid composition of strain IC-180^T was analysed by the MIDI system. It contained iso-C_{16:0} (56.0 %), anteiso-C_{17:0} (12.4 %), iso-C_{18:0} (11.4 %), iso-C_{17:0} (4.6 %), anteiso-C_{15:0} (4.1 %), iso-C_{17:1}ω5c (3.1 %), iso-C_{16:1}H (1.4 %), C_{17:0} (1.1 %), iso-C_{15:0} (1.1 %) and C_{16:0} (1.1 %). The menaquinone system and polar lipid composition were analysed as described by Minnikin *et al.* (1984). MK-9(H₈) was a major component of the menaquinone system. The phospholipid lipid fraction contained diphosphatidylglycerol, phosphatidyl-*N*-methylethanolamine, an unknown ninhydrin-positive phosphoglycolipid, phosphatidylinositol and phosphatidylinositolmannoside.

Total DNA was prepared from the isolate and DNA base composition was determined by the HPLC method as described by Tamaoka (1994). DNA G+C content was 74.1 ± 0.3 mol% (two determinations). The almost entire region of the 16S rRNA gene was amplified and sequenced as described previously (Namwong *et al.*, 2005). Of 1482 nt positions determined, 1225 positions were compared for reconstruction of the phylogenetic tree after alignment with related strains and uncultured clones by the program CLUSTAL X (Thompson *et al.*, 1997) followed by manual editing of the alignment. 16S rRNA gene sequence analysis revealed that strain IC-180^T was a member of the order *Acidimicrobiales*. Currently, there are two families in this order: *Acidimicrobiaceae*, represented by the three species *Acidimicrobium ferrooxidans*, *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* (Clark & Norris, 1996; Johnson *et al.*, 2009); and family *Iamiaceae*, represented by the single species *Iamia majanohamensis* (Kurahashi *et al.*, 2009). In the phylogenetic tree (Fig. 2), strain IC-180^T formed a coherent cluster with two uncultured bacteria, one from an acidic geothermal site in Yellowstone National Park, USA (clone W2bXIIb31), and the other from a moderately thermophilic bioreactor operated in China (clone BS-B54) with similarities of 99.4–99.5%. Other uncultured bacterial clones near this cluster (i.e. clones S5, BA84, SK299 and W2bXIb49) were also detected from acidic and/or thermophilic habitats. This cluster was distantly related to the other two clusters corresponding to the two families of the order, i.e.

Acidimicrobiaceae and *Iamiaceae*. Sequence similarities between strain IC-180^T and the three known *Acidimicrobiaceae* species ranged from 93.3 to 92.0% and the similarity value between strain IC-180^T and *Iamia majanohamensis* F12^T was 91.5%. The 16S rRNA signature pattern was the same as that of the family *Acidimicrobiaceae* as indicated by Zhi *et al.* (2009), except for 952:1229 (U–A).

The present study reveals that strain IC-180^T is an acidophilic, moderately thermophilic actinobacterium capable of dissimilatory reduction of ferric iron with H₂ or organic substances as electron donors for growth under anaerobic or autotrophic conditions. From the phylogenetic analysis, the strain can be placed in the order *Acidimicrobiales*; however, it is not affiliated with members of the two known families, *Acidimicrobiaceae* and *Iamiaceae*. The phenotypic properties of strain IC-180^T indicate that this isolate seems to be more related to species of the family *Acidimicrobiaceae*, which thrive in acidic, mineral-rich, moderately thermophilic habitats and are capable of dissimilatory iron metabolism. On the other hand, *Iamia majanohamensis*, the sole member of the family *Iamiaceae*, is a non-acidophile isolated from a sea cucumber. In addition, *Ilumatobacter fluminis*, whose family membership has not been clarified at present although it seems to be affiliated with the family *Iamiaceae*, inhabits marine sediments (Matsumoto *et al.*, 2009). Iron-oxidation capability has been demonstrated for known species of the family *Acidimicrobiaceae*, as well as the two strains of '*Acidithiomicrobium*', in pure or mixed

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain IC-180^T. *Frankia* sp. (L40622) was used as the outgroup. Numbers at nodes indicate bootstrap values based on 1000 resamplings (>500). Bar, 0.01 substitutions.

culture (Brierley, 1978; Clark & Norris, 1996; Johnson *et al.*, 2003, 2009; Davis-Belmar & Norris, 2009), but it was not observed for strain IC-180^T. *Ferrimicrobium acidiphilum* and *Ferrithrix thermotolerans* are obligate heterotrophs, whereas *Acidimicrobium ferrooxidans* and strain IC-180^T are capable of growing under autotrophic conditions at moderately thermophilic temperatures (optimum growth at 45–50 °C). Differential characteristics are summarized in Table 1. On the basis of the phenotypic, chemotaxonomic and phylogenetic properties, strain IC-180^T represents a novel species in a new genus in the order *Acidimicrobiales*. Due to the distant lineage of strain IC-180^T from the families *Acidimicrobiaceae* and *Iamiaceae*, this isolate may represent a new family; however, such a conclusion should not be drawn until other strains related to IC-180^T have been isolated and compared in detail with members of all genera of the order *Acidimicrobiales*. Here, the name *Aciditerrimonas ferrireducens* gen. nov., sp. nov. is proposed to accommodate strain IC-180^T; the type strain is IC-180^T (=JCM 15389^T =DSM 45281^T).

Description of *Aciditerrimonas* gen. nov.

Aciditerrimonas (A.ci.di.ter.ri.mo'nas. L. neut. n. *acidum* an acid; L. n. *terra* soil; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Aciditerrimonas* acidic soil monad).

Cells are short rods, 0.5–0.6 × 0.8–1.1 µm, motile with peritrichous flagella. Gram-positive. Do not form spores. Thermoacidophilic, growing optimally at 50 °C and pH 3.0. Facultatively anaerobic and autotrophic. Capable of reducing ferric iron. The peptidoglycan contains *meso*-diaminopimelic

acid. The major cellular fatty acids are iso-C_{16:0}, anteiso-C_{17:0} and iso-C_{18:0}. The major quinone component is MK-9(H₈). The phospholipid composition includes phosphatidyl-*N*-methylethanolamine and an unknown ninhydrin-positive phosphoglycolipid. Phylogenetically affiliated in the order *Acidimicrobiales* according to 16S rRNA gene sequence comparisons. The 16S rRNA signature pattern is the same as that of the family *Acidimicrobiaceae* as indicated by Zhi *et al.* (2009), except for 952:1229 (U–A). The type species is *Aciditerrimonas ferrireducens*.

Description of *Aciditerrimonas ferrireducens* sp. nov.

Aciditerrimonas ferrireducens (fer.ri.re.du'cens. L. n. *ferrum* iron; L. part. adj. *reducens* bringing back, leading back; N.L. part. adj. *ferrireducens* iron-reducing).

Morphological, cultural properties, chemotaxonomic and phylogenetic features are as described for the genus. Grows at 35–58 °C (optimally at 50 °C) and pH 2.0–4.5 (optimally at pH 3.0). Grows aerobically, or anaerobically in the presence of ferric iron. Heterotrophic growth occurs using yeast extract, glucose, lactose, mannose and xylose as carbon and energy sources. Autotrophic growth occurs by reducing ferric iron with hydrogen under anaerobic conditions. Does not oxidize ferrous iron.

The type strain is IC-180^T (=JCM 15389^T =DSM 45281^T), which was isolated from solfataric soil at Ohwaku-dani, Hakone, Japan. The DNA G + C content of the type strain is 74 mol%.

Table 1. Major characteristics that distinguish strain IC-180^T from known genera of the order *Acidimicrobiales*

Taxa: 1, strain IC-180^T (data from this study); 2, *Acidimicrobium* (data from Clark & Norris, 1996, except where marked); 3, *Ferrimicrobium* (data from Johnson *et al.*, 2009); 4, *Ferrithrix* (data from Johnson *et al.*, 2009); 5, *Iamia* (data from Kurahashi *et al.*, 2009); 6, *Ilumatobacter* (data from Matsumoto *et al.*, 2009). ND, No data available.

Characteristic	1	2	3	4	5	6
Cell shape	Short rod	Rod	Rod	Filament	Rod	Rod
Gram reaction	+	+	–	–	+	+
Motility	+	+	+	–	–	–
Growth temperature (°C)*	50	45–50	35	43	28–30	26–31
Growth pH*	3.0	2.0	2.0	1.8	7.0	7–11
Autotrophic growth	+	+	–	–	ND	ND
Oxidation of ferrous iron	–	+	+	+	ND	ND
Major fatty acids†	i-C _{16:0} , ai-C _{17:0} , i-C _{18:0}	i-C _{16:0} , ai-C _{17:0} ‡	i-C _{16:0} , ai-C _{15:0}	i-C _{16:0}	C _{17:0} , C _{17:1} ω8c	C _{16:0} , C _{17:1} ω9c, i-C _{17:0}
Major menaquinone	MK-9(H ₈)	MK-9(H ₈)‡	MK-8(H ₁₀)	ND	MK-9(H ₆)	MK-9(H ₈)
DNA G + C content (mol%)	74	67–69	55	50	74	68
Isolated from geothermal/mine habitat	+	+	+	+	–	–

*Optimum values are given, except for the genus *Ilumatobacter*, where the range is shown.

†Components present at more than 5% total fatty acids are given, except for the genera *Iamia* and *Ilumatobacter*, where components more than 10% are shown.

‡Data from this study.

Acknowledgements

This work was supported, in part, by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (no. 13660338).

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