Acidiplasma aeolicum gen. nov., sp. nov., a euryarchaeon of the family *Ferroplasmaceae* isolated from a hydrothermal pool, and transfer of *Ferroplasma cupricumulans* to *Acidiplasma cupricumulans* comb. nov.

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A novel acidophilic, cell-wall-less archaeon, strain V^T, was isolated from a hydrothermal pool on Vulcano Island, Italy. The morphology of cells was observed to vary from pleomorphic to coccoid. The temperature range for growth of strain V^T was 15–65 °C with an optimum at 45 °C. The pH for growth ranged from pH 0 to 4 with an optimal at pH 1.4–1.6. Strain V^T was able to grow aerobically and anaerobically, oxidizing ferrous iron and reducing ferric iron, respectively. The isolate grew chemo-organotrophically with yeast extract and yeast extract with glucose as the sources of energy and carbon. The molar G + C content in the DNA was 36 mol%. 16S rRNA gene sequence analysis demonstrated that strain V^T was a member of the family *Ferroplasmaceae*, order *Thermoplasmatales*, phylum *Euryarchaeota*, showing sequence identities of 100 % with *Ferroplasma cupricumulans* BH2^T, 95.4 % with *Ferroplasma acidiphilum* Y^T, 94 % with *Picrophilus torridus* DSM 9790^T and 92 % with *Picrophilus oshimae* DSM 9789^T. 16S rRNA gene sequence-based phylogenetic analysis showed that strain V^T formed a monophyletic cluster together with *F. cupricumulans* BH2^T and all other thermophilic isolates with available 16S rRNA gene sequences, whereas *F. acidiphilum* Y^T formed another cluster with mesophilic isolates within the family *Ferroplasmaceae*. DNA–DNA hybridization values between strain V^T and *F. cupricumulans* BH2^T were well below 70 %, indicating that the two strains belong to separate species. Principal membrane lipids of strain V^T were dibiphytanyl-based tetraether lipids containing pentacyclic rings. The polar lipids were dominated by a single phosphoglycolipid derivative based on a galactosyl dibiphytanyl phosphoglycerol tetraether, together with smaller amounts of monoglycosyl and diglycosyl dibiphytanyl ether lipids and the corresponding phosphoglycerol derivatives. The major respiratory quinones present were naphthoquinone derivatives. Given the notable physiological and chemical differences as well as the distinct phylogenetic placement of the new isolate relative to the type species of the genus *Ferroplasma*, we propose strain V^T as a member of a new genus and species, *Acidiplasma aeolicum* gen. nov.,

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain V^T is AM943980.

A growth curve for strain V^T is available as supplementary material with the online version of this paper.

sp. nov. The type strain of *Acidiplasma aeolicum* is strain V^T (=DSM 18409^T =JCM 14615^T). In addition, we propose to transfer *Ferroplasma cupricumulans* Hawkes *et al.* 2008 to the genus *Acidiplasma* as *Acidiplasma cupricumulans* comb. nov. (type strain BH2^T =DSM 16551^T =JCM 13668^T).

Members of the family *Ferroplasmaceae* inhabit a number of acidic environments in diverse geographical locations (Golyshina & Timmis, 2005) with stable, low pH, moderate temperatures and high concentrations of iron, e.g. sulfide ores, mining wastewaters and acidic pools. The family *Ferroplasmaceae* is one of the three families of the order *Thermoplasmatales*. Members of this order are the most acidophilic microbes known to date. *Thermoplasma acidophilum* (from a self-heating coal pile; Darland *et al.*, 1970) and *Thermoplasma volcanium* (isolated from submarine and continental solfataras on Vulcano Island; Segerer *et al.*, 1988), *Picrophilus torridus* and *Picrophilus oshimae* (Schleper *et al.*, 1995, 1996), *Ferroplasma acidiphilum* and *Ferroplasma cupricumulans* (Golyshina *et al.*, 2000; Hawkes *et al.*, 2006, 2008) and the recently described *Thermogymnomonas acidicola* (Itoh *et al.*, 2007) constitute the described species of the order *Thermoplasmatales* with validly published names. Based on 16S rRNA gene sequence data, the order *Thermoplasmatales* constitutes an isolated cluster, which branches in most phylogeny calculations between the *Methanobacteriales* and the *Methanomicrobiales*/halophiles (Huber & Stetter, 2006).

In the present work, we describe the isolation and characterization of a novel acidophilic archaeon from the order *Thermoplasmatales*, family *Ferroplasmaceae*, strain V^T, that belongs to a new genus and species, *Acidiplasma aeolicum* gen. nov., sp. nov., and comparison of this isolate with the type strain of *Ferroplasma cupricumulans* (Hawkes *et al.*, 2006), which we propose to transfer to the genus *Acidiplasma* as *Acidiplasma cupricumulans* comb. nov.

Water and sediment samples from a hydrothermal pool near Porto di Levante on Vulcano Island, in the Aeolian archipelago near Sicily, Italy, were taken on 30 April 2005 using sterile 50 ml Falcon tubes and transported to the laboratory at ambient temperature. The pH of the aqueous phase was measured on-site to be 2.0; the content of Fe²⁺ was 25–50 mg l⁻¹. Incorporations of sulfur in sand and gravel were visible, and the temperature on-site at the time of sampling was 25 °C. Samples of water-saturated volcanic sand/gravel were used as inocula (5 %, v/v) in medium 9K, pH 1.7, supplemented with 0.02 % yeast extract, in 0.5 l Erlenmeyer flasks with trace element solution (SL-10), as reported elsewhere (Golyshina *et al.*, 2000). The medium was additionally amended with potassium tetrathionate at concentrations of 0.5–20 mM. The flasks were incubated with agitation (120 r.p.m.) at temperatures of 30, 40 and 50 °C. Later, serial dilutions of enrichment cultures were carried out to isolate pure cultures of the micro-organisms. Culture purity was controlled by phase-contrast microscopy and by PCR where the major criteria were (i) the

inability to obtain any bacterial PCR amplicons with standard oligonucleotide pairs (F27–R518 and F530–R1492) and (ii) the homogeneity of sequences of PCR amplicons obtained using *Archaea*-specific primers (oligonucleotide sequences given below). The culture containing the isolate designated 'V' was selected for further investigations. To estimate the optimal growth parameters, the culture was incubated at 0–70 °C and pH 0–5.

F. cupricumulans BH2^T was cultured under the conditions recommended in the primary isolation/description report by Hawkes *et al.* (2006).

Exponentially grown cells were fixed with 1 % glutardialdehyde in growth medium at 4 °C for 14 days. Fixed cells were adsorbed to poly-L-lysine-treated Formvar carbon foils for 15 min at ambient temperature, which were then washed with water for 3 s, blotted with filter paper and air-dried. Samples were shadow-cast with Pt/C as described previously (Golyshina *et al.*, 2000). After initial fixation, cells were additionally fixed at a final concentration of 2 % glutardialdehyde in 100 mM cacodylate, pH 7.2. Cells were post-fixed with 1 % OsO₄/100 mM cacodylate, pH 7.2, for 90 min at ambient temperature, washed once for 2 min in 100 mM cacodylate, pH 7.2, and dehydrated in an ascending ethanol series before being embedded. The 70 % ethanol phase was supplemented with 1 % uranyl acetate. Cells were finally embedded in epoxy resin (Spurr, 1969). Energy-filtered transmission electron microscopy of ultrathin sections and shadow-cast samples was carried out according to Lünsdorf *et al.* (2001).

A general impression of the overall shape of cells of strain V^T and *F. cupricumulans* BH2^T could be obtained from shadow-cast preparation by transmission electron microscopy (Figs 1a and 2a). Strain V^T appeared to form compact pleomorphic to coccoid cells (Fig. 1a) with a tendency towards subsphericalization of the cell body under the growth conditions used (Fig. 1a; open arrow-heads). Corresponding ultrathin sections of cells showed cytoplasmic voids (Fig. 1b; asterisks). Cells were delineated by a unique cytoplasmic membrane [Figs 1b and 2b; cm (inset)] and no additional surface-coating matrix could be observed. Occasionally elongated, pleomorphic cells could be observed (Fig. 1a, b; p), but selenomorph cells were also present (Fig. 1a, b; single arrows). Cells were non-motile; mycoplasma-like gliding motility has not been observed by phase-contrast microscopy. A further morphological feature of strain V^T, which could not be observed for *F. cupricumulans* BH2^T, was the presence of filamentous protrusions extending from the cell surface [Fig. 1a, b (inset); twin-arrows].

Fig. 1. Ultrastructure of strain V^T after shadow-casting (a) and ultrathin-sectioning (b). Selenomorphous cells (single arrows) are a characteristic feature, but cells generally appear pleomorphic (p). Subspherulization (open arrowheads) is typically recognized, possibly based on the formation of intracellular voids (asterisks). The inset in (b) shows detailed features of typical cell morphology; the cytoplasmic membrane is indicated (cm). Double arrows indicate filamentous protrusions. The large arrowhead in (a) indicates the shadowing direction. C, Chromosome.

Cells of *F. cupricumulans* BH2^T appeared rather rod-like and tended to branch (Fig. 2a; double arrowheads and inset). Similarly to strain V^T , these cells contained electron-translucent intracellular voids (Fig. 2b; asterisks), which

Fig. 2. Shadow-cast (a) and ultrathin-sectioned (b) samples of *F. cupricumulans* BH2^T. See legend to Fig. 1 for further details. Double arrowheads indicate branched cells.

often tended to form extrusions (Fig. 2b; double arrows), and could finally form vesicles.

Growth was monitored by determination of the protein content of the culture using the Bio-Rad protein assay kit. Concentrations of Fe^{2+} were estimated using Merckoquant paper test strips (Merck). Pure cultures of strain V^T were grown routinely in 500 ml Erlenmeyer flasks on a rotary shaker (150 r.p.m.) at 42–45 °C containing 100 ml medium 9K with $FeSO_4 \cdot 7H_2O$ (25 g l^{-1}) and trace element

solution SL-10 (Golyshina *et al.*, 2000) or AB medium (Segeer *et al.*, 1988) supplemented with yeast extract (0.1 %, w/v) and glucose (0.1–1 %).

The optimal conditions for growth of strain V^T (see the growth curve in Supplementary Fig. S1, available in IJSEM Online) were in the ferrous iron-containing medium 9K supplemented with yeast extract (0.02 % w/v) with the addition of potassium tetrathionate (in the range 0.5–20 mM; best growth at 2–5 mM). The specific growth rate under these conditions was 0.032 h⁻¹. Strain V^T grew in the temperature range 15–65 °C, with an optimum at 42–45 °C, and at pH 0–4, with an optimum at pH 1.4–1.6. Strain V^T also grew reasonably well chemo-organotrophically (albeit slower, with the specific growth rate no higher than 0.02 h⁻¹) without addition of ferrous iron: on yeast extract alone, at 0.002–1 % (w/v), and on yeast extract and 0.1–1 % glucose in modified Allen medium (AB) (Segeer *et al.*, 1988).

Strain V^T exhibited no growth on elemental sulfur in AB medium. Growth of strain V^T without potassium tetrathionate was scarce, yielding 10-fold less biomass in total, and was accompanied by a very slow oxidation of ferrous iron [360 h for full conversion of Fe(II) to Fe(III)], which is in stark contrast to the full conversion within 72 h in the presence of potassium tetrathionate. However, the reduced sulfur compounds potassium tetrathionate and sodium thiosulfate alone did not support growth of strain V^T.

Aerobic growth on other organic substrates was determined using Biolog GN2 microplates with medium 9K and AB medium with and without 0.02 % yeast extract. Growth was estimated at 45 °C after incubation for 48 h (9K) and 96 h (AB). The substrates examined were as follows: sugars and related compounds: L-arabinose, fructose, sucrose, L-sorbitol, L- and D-glucose, glucose 1-phosphate, glucose 6-phosphate, maltose, D-xylose, D-mannitol, lactose, cellobiose, D-galactose, mannose, L-fucose, gentiobiose, *myo*-inositol, D-lactulose, melibiose, methyl β -D-glucoside, D-psicose, rhamnose, L-rhamnose, D-sorbitol, trehalose, turanose, xylitol, cyclodextrin, dextrin, inosine, uridine, thymidine and glycogen; organic acids and their salts: aminobutyric acid, methyl pyruvate, monomethyl succinate, acetic acid, *cis*-acetic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, urocanic acid and L-pyroglutamic acid; amino acids: glucuronamide, alaninamide, D-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, D-serine, D-serine, L-threonine, DL-carnitine, putrescine and phenylethylamine; alcohols: 2-aminoethanol,

2,3-butanediol, glycerol, L- α -glycerol phosphate, adonitol, D-arabitol and i-erythritol; and others: Tweens 40 and 80, N-acetyl D-galactosamine and N-acetyl D-glucosamine. Strain V^T was not capable of growth on any of the organic substrates included in the GN2 microplate under any conditions tested with the sole exception of utilization of glucose in the presence of yeast extract.

Anaerobic growth was assayed in closed vessels (100 ml) with medium 9K with Fe₂(SO₄)₃ as electron acceptor, supplemented with 0.02 % (w/v) yeast extract and potassium tetrathionate (5 mM) as electron donor, and in AB medium with yeast extract (0.02–0.1 % w/v). The medium was flushed with N₂/CO₂ (80:20, v/v) or pure N₂ and the headspace contained the same gas mixture used for flushing. Strain V^T demonstrated anaerobic growth in all cases. With only N₂ present in the headspace, strain V^T grew on yeast extract, suggesting it is able to perform fermentation.

Inhibition of growth of strain V^T under optimal growth conditions was observed for rifampicin, chloramphenicol and tetracycline; no inhibition was observed with streptomycin, kanamycin, vancomycin, gentamicin or ampicillin (all antibiotics added at 100 μ g ml⁻¹).

Lipids were extracted using a standard two-stage protocol. Briefly, respiratory lipoquinones were extracted using methanol/hexane (2:1, v/v) and extracted into the hexane phase by the addition of 1 vol. cold hexane. The methanolic aqueous phase was further re-extracted by the addition of 2 vols cold 0.3 % aqueous NaCl followed by 2 vols cold hexane. The pooled hexane phases were combined and concentrated to a small volume under a stream of nitrogen. After separation by TLC into lipoquinone classes (e.g. naphthoquinones, benzoquinones), the respiratory lipoquinone composition of each class was determined by HPLC, using an RP₁₈ column and heptane/methanol (1:9, v/v) as a solvent.

Polar lipids were further extracted from the cell debris and the aqueous methanolic phase by bringing the solvent to a final chloroform/methanol/0.3 % NaCl ratio of 1:2:0.8 (by vol.). Cell debris was removed by centrifugation and lipids were extracted from the supernatant into chloroform by the addition of chloroform and aqueous 0.3 % NaCl to give a final chloroform/methanol/0.3 % NaCl ratio of 1:1:0.9 (by vol.). Polar lipids were dried under a stream of nitrogen and redissolved in a small volume of chloroform/methanol (2:1, v/v). Polar lipids were separated on silica gel thin layers (Fig. 3) using the solvent systems chloroform/methanol/water (65:25:4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) in the second dimension. Total lipids were visualized using 5 % molybdotetraphosphoric acid in ethanol. Functional groups were detected with Zinzadze reagent (phosphate), α -naphthol/sulfuric acid (sugars), ninhydrin (free amino groups) and periodate-Schiff (vicinal hydroxyl groups).

Fig. 3. Two-dimensional TLC of polar lipids of strain V^T and *Thermoplasma acidophilum* DSM 1728^T. The solvent system is detailed in the text. PL, Phospholipid (probably phosphatidic acid); PG, phosphatidylglycerol; PGL1, glycosyl-dibiphytanyl-phosphoglycerol; PGL2 and PGL3, phosphoglycolipids (respectively containing two and three sugar residues); GL1, GL2 and GL3, glycolipids (respectively containing one, two and three sugar residues).

MALDI MS was carried out on lipids isolated from unsprayed TLC plates that had been eluted from the silica, on a Bruker Ultraflex instrument (Bruker-Daltonics) using a matrix of α -cyano-4-hydroxycinnamic acid. For MS-MS analyses, selected parent ions were subjected to laser-induced dissociation (LID) and the resulting fragment ions were separated by the second TOF stage (LIFT) of the instrument. Carbohydrate compositional analyses were performed according to Chaplin (1982). The carbohydrate moiety in the major glycosyl dibiphytanyl phosphoglycerol lipid of strain V^T was different from those of representatives of the genera *Thermoplasma*, *Picrophilus* and *Ferroplasma* reported previously (Schleper *et al.*, 1996; Swain *et al.*, 1997; Batrakov *et al.*, 2002) (see below for more details).

The G + C content of the genomic DNA was 36 mol%, as determined directly by HPLC with a Zorbax Eclipse XDB-C8 column (Agilent), with purified, non-methylated lambda phage DNA (Sigma) used as a control, according to methods described previously (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984).

To determine the 16S rRNA gene sequence, total DNA was isolated from a late-exponential phase culture by using the GNOME DNA kit (Qbiogene). The 16S rRNA gene was amplified by PCR using the forward primers A23F (5'-TCYGGTTGATCCTGCC-3') and F530 (5'-TCCGTGCCAGCAGCCGCGC-3') and the reverse primer R1492 (5'-CGGYTACCTTGTTACGACTT-3'). Amplification was done in 50 μ l reaction volumes with *Taq* DNA polymerase (Invitrogen) and original reagents according to the basic PCR protocol. The amount of template DNA used was approximately 0.5 ng. The reaction ran for 30 cycles, each of 96 °C for 1 min, 45 °C for 1 min and 72 °C for 2 min, with a final extension at 72 °C for 10 min. Sequencing of the gel-purified PCR product was performed with the above oligonucleotides according to the protocol for the BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems). The GenBank NR database was searched for similar sequences using the online BLASTN program (Altschul *et al.*, 1997). Phylogenetic and molecular evolutionary analyses were conducted using multiple

alignment CLUSTAL W software at the EBI website (<http://www.ebi.ac.uk>) together with the phylogeny analysis software MEGA version 3.1 (Kumar *et al.*, 2004). The latter was used for phylogenetic distance calculations and tree construction (neighbour-joining method, Jukes-Cantor model, with the sampling of 1000 tree replicates for bootstrap calculations).

The 16S rRNA gene sequence of strain V^T was observed to cluster within the family *Ferroplasmaceae*, with 95.4 % sequence identity to *F. acidiphilum* Y^T and 100 % sequence identity to *F. cupricumulans* BH2^T.

DNA-DNA hybridization of strain V^T with *F. cupricumulans* BH2^T was performed by the DSMZ Identification Service. Total DNA was isolated using a French pressure cell (Thermo Spectronic) from 3 g wet cell mass suspended in 2-propanol/water (1:1, v/v) and purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA-DNA hybridization was carried out as described by De Ley *et al.* (1970) with the modifications described by Huß *et al.* (1983) using a Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 $\times$ 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The analysis showed the level of DNA-DNA hybridization in 2 $\times$ SSC at 61 °C to be 46.4 and 53.1 % in two independent measurements, indicating that the strains tested do not belong to the same species according to the recommendations of Wayne *et al.* (1987).

16S rRNA gene sequence analysis of strain V^T revealed its affiliation with *F. cupricumulans* BH2^T (100 % 16S rRNA gene sequence identity) and its next-closest relatives to be *F. acidiphilum* Y^T, *Picrophilus torridus* DSM 9790^T and *P. oshimae* DSM 9789^T, with 95.4, 94 and 92 % 16S rRNA gene sequence identity, respectively (Fig. 4). The study of the physiology of strain V^T has indicated a number of features that are typical of members of the order *Thermoplasmatales* (acidophily, yeast extract requirement, aerobic and anaerobic lifestyles and ferrous iron as the substrate, which is, in particular, common to representatives of the family *Ferroplasmaceae*), but also showed a

Fig. 4. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strain V^T within the order *Thermoplasmatales*. Organisms with validly published names are indicated in bold. GenBank accession numbers are given in parentheses. Two distinct monophyletic clusters, one including thermophilic isolates (strain V^T, BH2^T, JTC3 and L1) and another comprising mesophilic isolates (*F. acidiphilum* Y^T, DR1, fer1 and MT17), are well separated. The sequence of *Methanosarcina barkeri* DSM 800^T was used as the outgroup. Bar, 0.05 substitutions per nucleotide position.

number of distinct features (Table 1). The optimal temperature for growth of strain V^T (42–45 °C) is higher than that of *F. acidiphilum* Y^T (35 °C) and is within the lower growth temperature limit for thermophiles. Furthermore, the phylogenetic analysis affiliated strain V^T with the archaeon *F. cupricumulans* BH2^T and all other thermophilic isolates for which 16S rRNA gene sequences are available in GenBank (JTC3 and L1) and which form a separate cluster within the family *Ferropasmaceae* (Fig. 4). In contrast, the type strain of the type species of *Ferroplasma*, *F. acidiphilum* Y^T, clusters with the mesophilic isolates DR1, fer1 and MT17 (Edwards *et al.*, 2000; Dopson *et al.*, 2004; Golyshina & Timmis, 2005). Physiologically and phylogenetically, these organisms, representing a cluster of thermophiles within the family *Ferropasmaceae*, occupy an interim position between the common, mesophilic members of the family *Ferropasmaceae* and the thermophiles of the family *Picrophilaceae*.

The upper temperature limit for growth of strain V^T (about 65 °C) was close to that of moderately thermophilic members of the genera *Thermoplasma* and *Picrophilus*, but the optimum is closer to all known *Ferroplasma*-related

strains. The isolate was able to grow over relatively wide ranges of temperature (15–65 °C) and pH (0–4). Such a broad tolerance suggests that the organism is well adapted to environmental changes and might reflect temperature/pH fluctuations at its isolation site.

We also performed MALDI-TOF analysis of the proteome of strain V^T. Unlike *F. acidiphilum* Y^T, whose proteins have always shown the best hits to those from the '*Ferroplasma acidarmanus*' *in silico* proteome (Ferrer *et al.*, 2007), the majority of over 450 individual proteins resolved from strain V^T were not affiliated, according to peptide fragment masses, to any organism with a sequenced genome, with only a small fraction (<15 %) giving best hits to proteins from *Picrophilus torridus* (O. V. Golyshina, M. Ferrer, A. Belouqui and P. N. Golyshin, unpublished). These data further emphasize the low similarity in individual protein sequences of *F. acidiphilum* Y^T and strain V^T and point to very different genomic backgrounds.

Strain V^T is the first organism among the known members of the family *Ferropasmaceae* able to grow chemo-organotrophically on yeast extract or a mixture of yeast extract and glucose, producing a significant amount of

Table 1. Growth conditions and DNA G+C contents of organisms from the family *Ferropasmaceae* and representatives of other known genera of the order *Thermoplasmatales*

Taxa: 1, *F. acidiphilum* Y^T (data from Golyshina *et al.*, 2000); 2, *F. cupricumulans* BH2^T (Hawkes *et al.*, 2006); 3, strain V^T (this study); 4, *Picrophilus* (Schleper *et al.*, 1995); 5, *Thermoplasma* (Darland *et al.*, 1970; Huber & Stetter, 2006); 6, *Thermogymnomonas* (Itoh *et al.*, 2007).

Characteristic	1	2	3	4	5	6
Anaerobic growth	–	+	+	–	+	–
Fe ²⁺ oxidation	+	+	+	–	–	–
Chemo-organotrophic growth	–	–	+	+	+	+
Growth temperature (°C)						
Range	15–45	22–63	15–65	47–65	33–67	38–68
Optimum	35	53.6	45	60	59	60
pH for growth						
Range	1.3–2.2	0.4–1.8	0–4	0–3.5	0.5–4.0	1.8–4
Optimum	1.7	1–1.2	1.4–1.6	0.7	1–2	3.0
DNA G+C content (mol%)	37	34	36	36	38–46	56

biomass (more than 100 mg protein l⁻¹), with maximal values after prolonged periods of time. Other simple organic substrates were not utilized by this organism. All known organisms belonging to the order *Thermoplasmales* have an absolute requirement for yeast extract for growth (Darland *et al.*, 1970; Schleper *et al.*, 1995, 1996; Golyshina *et al.*, 2000; Dopson *et al.*, 2004; Hawkes *et al.*, 2006; Itoh *et al.*, 2007).

Despite sharing 100% 16S rRNA gene sequence identity, strain V^T and *F. cupricumulans* BH2^T, isolated from an industrial mineral sulfide bioleaching heap in Monywa, Burma (Hawkes *et al.*, 2006), do not belong to the same species on the basis of DNA–DNA hybridization results.

Morphological differences between strain V^T and *F. cupricumulans* BH2^T were relatively clear: strain V^T forms pleomorphic to coccoid cells, while cells of *F. cupricumulans* BH2^T were characterized by rod-like forms with a tendency towards branched morphologies. A further morphological feature of strain V^T, distinct from *F. cupricumulans* BH2^T, is the presence of filamentous protrusions extending from the cell surface.

The major polar lipids present were compared with lipids extracted from *Thermoplasma acidophilum* DSM 1728^T. The major glycopospholipids present in strain V^T and *Thermoplasma acidophilum* DSM 1728^T had slightly different R_f values, indicating differences that probably result from differences in sugar components. Both compounds were rapidly periodate–Schiff-positive, indicating the liberation of formaldehyde and the presence of (at least) two adjacent hydroxyl groups, with one in a chain-terminal position. The R_f values of the glycolipids were consistent with those of mono-, di- and triglycosyl lipids. The R_f values of the major phospholipids, which were also rapidly periodate–Schiff-positive, were consistent with their identification as phosphoglycerol derivatives of the corresponding mono-, di- and triglycosyl glycolipids.

The most abundant phosphoglycolipid spot of strain V^T yielded a series of molecular ions exhibiting mass increments of 2 Da starting at *m/z* 1632.3 and ending at 1640.3 [M + Na]⁺ plus an analogous pattern 22 Da upmass [M – H + 2Na]⁺, suggesting the presence of dibiphytanyl phosphoglycerol tetraether compounds differing in the number (0–4) of cyclopentyl rings, with one free glycerol hydroxyl group linked to a hexose residue and the other via a phosphodiester linkage to another glycerol residue. Comparison of the glycopospholipid of strain V^T with the analogous structure isolated from *Thermoplasma acidophilum* DSM 1728^T revealed clear differences in the pattern of cyclopentyl rings and indicated, on average, more cyclopentyl rings in the former organism. LID experiments yielded fragment ions that confirmed the presence of a dibiphytanyl phosphoglycerol tetraether basic structure modified by a phosphatidylglycerol residue linked to one free hydroxyl group and a hexose residue linked to the other. The identity of this hexose residue in the major lipid from both strains was determined by GC/MS after

methanolysis followed by trimethylsilylation. Whereas gulose was identified in the lipid from *Thermoplasma acidophilum* DSM 1728^T, galactose was found in the analogous compound from strain V^T, clearly demonstrating the differences between the two strains.

NMR analysis of the major polar lipid from *Thermoplasma acidophilum* DSM 1728^T was consistent with the results reported by Swain *et al.* (1997). NMR analysis of PGL1 (Fig. 3) of strain V^T showed that the sugar present is β-galactopyranose, consistent with the finding of galactopyranose after hydrolysis of the lipid. No evidence was found for the presence of points of unsaturation of the isoprenoid side chain. Full details will be reported elsewhere.

The predominant respiratory lipoquinones present were naphthoquinones that were present in two distinct bands. They did not co-elute on an RP₁₈ column with authentic MK-7, MMK-7 (thermoplasma quinone 7) or MTK-7 (menathioquinone-7), which have been reported previously for members of this order investigated to date (Collins, 1985; Shimada *et al.*, 2001).

Based on other reported data, it is interesting to note that members of the genus *Thermoplasma* have β-L-gulose in this lipid (Swain *et al.*, 1997), whereas members of the genera *Picrophilus* and *Ferroplasma* are reported to have β-D-glucose in their major glycosyl dibiphytanyl phosphoglycerol lipid. These differences also correlate with the groupings found by 16S rRNA gene sequence analysis, and one may consider them to have both evolutionary and taxonomic significance.

On the basis of 16S rRNA gene sequence comparison (95.4% sequence identity with *F. acidophilum* Y^T and its clustering into a very distinct lineage with zero sequence mismatches) and physiological (in particular, its thermophilic nature) and chemical (the difference in sugar components in its major glycosyl dibiphytanyl phosphoglycerol lipids) characteristics and, finally, the lack of similarity in peptide fragment masses in the whole-cell proteome, which suggests a stark difference at the genomic level, strain V^T is proposed to represent a new genus and species, *Acidiplasma aeolicum* gen. nov., sp. nov., within the family *Ferroplasmaceae*. Furthermore, the genus *Acidiplasma* is herewith proposed to accommodate another species, *Acidiplasma cupricumulans* comb. nov., represented by strain BH2^T, previously described as *Ferroplasma cupricumulans* Hawkes *et al.* 2008.

Description of *Acidiplasma* gen. nov.

Acidiplasma (A.ci.di.plas' ma. N.L. neut. n. *acidum* an acid; Gr. neut. n. *plasma* something shaped or moulded; N.L. neut. n. *Acidiplasma* an acid-living form).

Cells are irregular cocci, varying from spherical to filamentous. Facultatively anaerobic. Acidophilic. Moderately thermophilic. Oxidize ferrous iron in the presence of trace amounts of yeast extract. 16S rRNA gene sequences group within the family *Ferroplasmaceae*, in the

order *Thermoplasmatales*, phylum *Euryarchaeota*. Known habitats are hydrothermal pools and chalcocite-containing sulfide ores. The type species is *Acidiplasma aeolicum*.

Description of *Acidiplasma aeolicum* sp. nov.

Acidiplasma aeolicum (ae.o'li.cum. L. neut. adj. *aeolicum* from the Aeolian archipelago, to which Vulcano Island belongs, where the type strain was isolated).

Displays the following properties in addition to those described for the genus. Exhibits filamentous protrusions extending from the cell surface. Temperature and pH ranges for growth are 15–65 °C (optimum 42–45 °C) and pH 0–4 (optimum pH 1.4–1.6). Oxidizes reduced sulfur species. Able to grow organoheterotrophically with yeast extract and glucose. The G+C content of DNA of the type strain is 36 mol%. Principal membrane lipids are dibiphytanyl-based tetraether lipids. The polar lipids are dominated by a single phosphoglycolipid derivative based on a galactosyl dibiphytanyl phosphoglycerol tetraether, together with smaller amounts of mono- and diglycosyl dibiphytanyl ether lipids and the corresponding phosphoglycerol derivatives. The major respiratory quinones present are naphthoquinone derivatives.

The type strain is strain V^T (=DSM 18409^T = JCM 14615^T), which was isolated from a hydrothermal pool on Vulcano Island, Italy.

Description of *Acidiplasma cupricumulans* (Hawkes et al. 2008) comb. nov.

Acidiplasma cupricumulans (cu.pri.cu'mu.lans. L. neut. n. *cuprum* copper; L. part. adj. *cumulans* heaping up, accumulating; N.L. part. adj. *cupricumulans* copper-accumulating).

Basonym: *Ferroplasma cupricumulans* Hawkes et al. 2008.

The description is the same as that given for *Ferroplasma cupricumulans* by Hawkes et al. (2006). Morphology is as described for the genus; in addition, cells are often rod-like, with a tendency to form branched morphologies. The type strain is strain BH2^T (=DSM 16551^T = JCM 13668^T).

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