**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 V7, 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 V7 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 V7 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 V7 was a member of the family *Ferroplasmaceae*, order *Thermoplasmatales*, phylum *Euryarchaeota*, showing sequence identities of 100% with *Ferroplasma cupricumulans* BH27, 95.4% with *Ferroplasma acidiphilum* YT7, 94% with *Picrophilus torridus* DSM 97907 and 92% with *Picrophilus oshimae* DSM 97897. 16S rRNA gene sequence-based phylogenetic analysis showed that strain V7 formed a monophyletic cluster together with *F. cupricumulans* BH27 and all other thermophilic isolates with available 16S rRNA gene sequences, whereas *F. acidiphilum* YT7 formed another cluster with mesophilic isolates within the family *Ferroplasmaceae*. DNA–DNA hybridization values between strain V7 and *F. cupricumulans* BH27 were well below 70%, indicating that the two strains belong to separate species. Principal membrane lipids of strain V7 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 V7 as a member of a new genus and species, *Acidiplasma aeolicum* gen. nov., sp. nov.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain V7 is AM943980.

A growth curve for strain V7 is available as supplementary material with the online version of this paper.
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., 2000, 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†, 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 Fe$^{3+}$ 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† was cultured under the conditions recommended in the primary isolation/description report by Hawkes et al. (2006).

Exponentially grown cells were fixed with 1 % glutaraldehyde 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 % glutaraldehyde in 100 mM cacodylate, pH 7.2. Cells were post-fixed with 1 % OsO$_4$/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 Lu¨nsdorf et al. (2001).

A general impression of the overall shape of cells of strain V† and *F. cupricumulans* BH2† could be obtained from shadow-cast preparation by transmission electron microscopy (Figs 1a and 2a). Strain V† 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 arrowheads). 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 selenomorphic 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†, which could not be observed for *F. cupricumulans* BH2†, was the presence of filamentous protrusions extending from the cell surface [Fig. 1a, b (inset); twin-arrowheads].
Cells of \textit{F. cupricumulans} BH2\textsuperscript{T} appeared rather rod-like and tended to branch (Fig. 2a; double arrowheads and inset). Similarly to strain V\textsuperscript{T}, these cells contained electron-translucent intracellular voids (Fig. 2b; asterisks), which 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\textsuperscript{2+} were estimated using Merckoquant paper test strips (Merck). Pure cultures of strain V\textsuperscript{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\textsubscript{4}.7H\textsubscript{2}O (25 g l\textsuperscript{-1}) and trace element
The optimal conditions for growth of strain VT (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) and 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 VT 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 VT 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) (Segerer et al., 1988).

Strain VT exhibited no growth on elemental sulfur in AB medium. Growth of strain VT 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 VT.

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-xylene, 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-glucaric acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylacetic acid, itaconic acid, κ-ketobutyric acid, α-ketoglutaric acid, κ-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-prolylglutamic acid; amino acids: gluturonamide, alaninamide, D-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycy1 L-aspartic acid, glycy1 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 VT 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 VT demonstrated anaerobic growth in all cases. With only N₂ present in the headspace, strain VT grew on yeast extract, suggesting it is able to perform fermentation.

Inhibition of growth of strain VT 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 μg 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 RF₁₈ 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% molybdatophosphoric acid in ethanol. Functional groups were detected with Zinnzade reagent (phosphate), α-naphthol/sulfuric acid (sugars), ninhydrin (free amino groups) and periodate–Schiff (vical hydroxyl groups).
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 x-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\textsuperscript{T} was different from that 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'-TCGGTTGATCCTGCC-3') and E530 (5'-TCCGTG-CCAGCAGCCCG-3') and the reverse primer R1492 (5'-CGGYTACCTTGTTACGACTT-3'). Amplification was done in 50 µl 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\textsuperscript{T} was observed to cluster within the family *Ferroplasmaceae*, with 95.4% sequence identity to *F. acidiphilum* V\textsuperscript{T} and 100% sequence identity to *F. cupricumulans* BH2\textsuperscript{T}.

DNA–DNA hybridization of strain V\textsuperscript{T} with *F. cupricumulans* BH2\textsuperscript{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 6×6 multiecell changer and a temperature controller with in situ temperature probe (Varian). The analysis showed the level of DNA–DNA hybridization in 2× 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\textsuperscript{T} revealed its affiliation with *F. cupricumulans* BH2\textsuperscript{T} (100% 16S rRNA gene sequence identity) and its next-closest relatives to be *F. acidiphilum* V\textsuperscript{T}, *Picrophilus torridus* DSM 9790\textsuperscript{T} and *P. oshimae* DSM 9789\textsuperscript{T}, with 95.4, 94 and 92% 16S rRNA gene sequence identity, respectively (Fig. 4). The study of the physiology of strain V\textsuperscript{T} has indicated a number of features that are typical of members of the order *Thermoplasmales* (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
The optimal temperature for growth of strain VT (42–45°C) is higher than that of *F. acidiphilum* YT (35°C) and is within the lower growth temperature limit for thermophiles. Furthermore, the phylogenetic analysis affiliated strain VT with the archaeon *F. cupricumulans* BH2 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 *Ferroplasmaceae* (Fig. 4).

In contrast, the type strain of the type species of *Ferroplasma*, *F. acidiphilum* YT, 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 *Ferroplasmaceae*, occupy an interim position between the common, mesophilic members of the family *Ferroplasmaceae* and the thermophiles of the family *Picrophilaceae*.

The upper temperature limit for growth of strain VT (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 VT. Unlike *F. acidiphilum* YT, 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 VT 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. Beloqui and P. N. Golyshin, unpublished). These data further emphasize the low similarity in individual protein sequences of *F. acidiphilum* YT and strain VT and point to very different genomic backgrounds.

Strain VT is the first organism among the known members of the family *Ferroplasmaceae* able to grow chemoorganotrophically on yeast extract or a mixture of yeast extract and glucose, producing a significant amount of

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

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Anaerobic growth</td>
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<td>−</td>
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<tr>
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<td>34</td>
<td>36</td>
<td>36</td>
<td>38–46</td>
<td>56</td>
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</table>
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 VT 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 VT and *F. cupricumulans* BH2\(^T\) were relatively clear: strain VT 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 VT, 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 glycolipids present in strain VT 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 a phosphoglycerol derivatives of the corresponding mono-, di- and triglycosyl glycolipids.

The most abundant phosphoglycolipid spot of strain VT 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 dibiphytanylglycerol 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 glycolipid spot of strain VT 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 dibiphytanylglycerol tetraether basic structure modified by a phophatidylglycerol 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 methanalysis 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 VT\(^T\), 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 VT\(^T\) 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 RP\(_{18}\) 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* VT\(^T\) 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 VT\(^T\) is proposed to represent a new genus and species, *Acidiplasma aeolicum* gen. nov., sp. nov., within the family Ferroplasmaceae. Furthermore, the genus *Acidiplasma* is hereewith 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 RNA 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).


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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References


