Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea

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An isolate of an acidophilic archaeon, strain Y^T, was obtained from a bioleaching pilot plant. The organism oxidizes ferrous iron as the sole energy source and fixes inorganic carbon as the sole carbon source. The optimal pH for growth is 1.7, although growth is observed in the range pH 1.3 to 2.2. The cells are pleomorphic and without a cell wall. 16S rRNA gene sequence analysis showed this strain to cluster phylogenetically within the order 'Thermoplasmatales' sensu Woese, although with only 89.9 and 87.2% sequence identity, respectively, to its closest relatives, *Picrophilus oshimae* and *Thermoplasma acidophilum*. Other principal differences from described species of the 'Thermoplasmatales' are autotrophy (strain Y^T is obligately autotrophic), the absence of lipid components typical of the 'Thermoplasmatales' (no detectable tetraethers) and a lower temperature range for growth (growth of strain Y^T occurs between 15 and 45 °C). None of the sugars, amino acids, organic acids or other organic compounds tested was utilized as a carbon source. On the basis of the information described above, the name *Ferroplasma acidiphilum* gen. nov., sp. nov. is proposed for strain Y^T within a new family, the *Ferroplasmaceae* fam. nov. Strain Y^T is the type and only strain of *F. acidiphilum*. This is the first report of an autotrophic, ferrous-iron-oxidizing, cell-wall-lacking archaeon.

Keywords: *Archaea*, 'Thermoplasmatales', acidophilic, chemolithoautotrophic, ferrous-iron-oxidizing

INTRODUCTION

Acidophilic aerobic or facultatively anaerobic *Archaea* that colonize biotopes such as pyrite ores, solfatara fields etc., where sulfur and iron are typically in reduced forms, generally represent two different phylogenetic groups of the *Archaea*, the order 'Thermoplasmatales' or 'Thermoplasmatales', which has been

Abbreviations: CID, collision-induced dissociation; FAB, fast-atom bombardment; MS, mass spectrometry.

The EMBL accession number for the 16S rRNA gene sequence of *Ferroplasma acidiphilum* strain Y^T is AJ224936.

proposed (Woese, 1987; Woese *et al.*, 1990; Segerer & Stetter, 1992b), but has not been validly published, and the order *Sulfolobales* (Segerer & Stetter, 1992a). These groups of acidophiles differ with respect to their phenotypic properties; first of all, with respect to the carbon and energy sources utilized. Some representatives of the *Sulfolobales*, e.g. *Acidianus brierleyi* (Segerer & Stetter, 1992a), members of the genus *Metallosphaera* (Fuchs *et al.*, 1995; Huber *et al.*, 1989) and *Sulfolobus hakonensis* (Takayanagi *et al.*, 1996), obtain energy by oxidizing sulfur, sulfide minerals and ferrous iron. Other species of the genus *Sulfolobus*, e.g. *Sulfolobus acidocaldarius* (Brock *et al.*, 1972) and

Sulfolobus solfataricus (Brierley & Brierley, 1973), utilize sulfur and reduced sulfur compounds. *Sulfolobus metallicus* (Huber & Stetter, 1991) exploits sulfidic ores, such as pyrite, sphalerite and chalcopyrite, and elemental sulfur as energy sources. Although other members of the *Sulfolobales* are able to grow chemolithoautotrophically, *S. metallicus* (Huber & Stetter, 1991) and *Acidianus ambivalens* (Fuchs *et al.*, 1996) are the only obligately chemolithoautotrophic species known. In contrast, species of both genera of the order 'Thermoplasmatales' described to date, *Thermoplasma* (Darland *et al.*, 1970; Segerer *et al.*, 1988; Segerer & Stetter, 1992b) and *Picrophilus* (Schleper *et al.*, 1995, 1996), are heterotrophic archaea that probably consume the decomposition products of the primary producers in solfatara fields and coal refuse piles, such as species of the genera *Acidianus*, *Thiobacillus* and *Sulfolobus*.

Here, we report the isolation, phylogenetic characterization and phenotypic characteristics of strain Y^T, isolated from a pyrite-leaching pilot plant and representing a hitherto undescribed species of a new genus that represents a new family within the order 'Thermoplasmatales'. Strain Y^T represents the only strictly autotrophic, cell-wall-deficient archaeon described to date. In recognition of its ability to oxidize ferrous iron and the absence of a distinct cell wall, together with the acidic origin of isolation, the name *Ferroplasma acidiphilum* gen. nov., sp. nov., within the family *Ferroplasmaceae* fam. nov., is proposed, and strain Y^T (= DSM 12658^T) is designated as the type strain.

METHODS

Isolation. Strain Y^T was isolated by serial dilution of the aqueous phase of a bioreactor of a pilot plant (Tula, Russia), which was bioleaching a gold-containing arsenopyrite/pyrite ore concentrate from Bakyrchik (Kazakhstan), in a modified 9K medium (see below). The temperature of the isolation source was 28–30 °C and the pH was 1.6–1.9. The purity of the culture and the absence of associated microorganisms were controlled directly by phase-contrast microscopy as well as by inoculation of heterotrophic liquid media. The purity of the culture was also estimated by (i) the inability to obtain any bacterial PCR amplicons and (ii) the homogeneity of sequences of PCR amplicons obtained by using *Archaea*-specific oligonucleotide primers.

Growth conditions. If not stated otherwise, strain Y^T was cultivated in 250 ml flasks with 100 ml modified medium 9K (Silverman & Lundgren, 1959) containing (l⁻¹): 0.4 g MgSO₄·7H₂O, 0.2 g (NH₄)₂SO₄, 0.1 g KCl, 0.1 g K₂HPO₄ and 25 g FeSO₄·7H₂O. The medium was supplemented with 0.02 % yeast extract (Difco) and trace elements, as described previously (Segerer & Stetter, 1992a). The pH of the medium was adjusted to 1.7 by adding 10 % (v/v) H₂SO₄ and measured with an InLab 416 electrode (Mettler Toledo). Different dilutions of HCl served as references for low pH values. Strain Y^T was cultivated on a rotary shaker (150 r.p.m.) at 35 °C. The 1000× vitamin stock solution contained (l⁻¹): 100 mg biotin, 350 mg nicotinic acid amide, 300 mg thiamin/HCl, 200 mg *p*-aminobenzoic acid, 100 mg pyridoxal hydrochloride, 100 mg calcium pantothenate and 50 mg vitamin B₁₂.

Anaerobic growth was assayed in closed vessels with or without FeSO₄ in the presence of acetic acid (0.2 %). The atmosphere consisted of 180 kPa CO₂ with or without the addition of 40 kPa H₂. Growth was monitored by the determination of the protein content of the culture using the Bio-Rad protein assay. The concentrations of Fe²⁺ and Fe³⁺ were determined by trilonometric titration (Reznikov *et al.*, 1970). Elemental sulfur and minerals containing reduced sulfur, Fe₂S, ZnS, PbS and Sb₂S₃, were sterilized by autoclaving and added to the medium.

Antibiotic-sensitivity analysis. The sensitivity of strain Y^T to antibiotics was determined by their addition in controlled concentrations into cultures that had been pre-grown for one generation in the medium outlined above.

Growth on organic substrates. The following organic compounds were tested as possible substrates at concentrations of 0.1–0.2 %, with or without the addition of FeSO₄. Growth was estimated, as described above, after incubation for 48 h. Sugars and related compounds: L-arabinose, fructose, sucrose, L-sorbitol, L- and D-glucose, glucose 1-phosphate, glucose 6-phosphate, L-maltose, D-xylose, D-mannitol, lactose, cellobiose, D-galactose, mannose, L-fucose, gentiobiose, *m*-inositol, lactulose, D-melibiose, β-methyl D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, D-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-pyrogutamic acid. Amino acids: glutaric acid, 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, L-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. Others: Tweens 40 and 80, *N*-acetyl D-galactosamine and *N*-acetyl D-glucosamine.

Electron microscopy. Vegetative cells were fixed in 2.5 % glutaraldehyde solution and absorbed to Formvar-coated copper grids (300 square mesh) for 20–90 s, depending on the cell density, blotted with filter paper and air-dried. Samples were shadowed unidirectionally with Pt/C at 15° angle of elevation and a final thickness of 4 nm in an MED 020 evaporation unit (Baltec). Negative staining, embedding and ultrathin sectioning were done according to methods described previously (Yakimov *et al.*, 1998).

Incorporation of labelled CO₂. Cells that had been pre-grown for 3 d in modified medium 9K under the standard conditions described above were collected from 500 ml culture, washed and resuspended in 1 ml of the same medium. Aliquots of 0.25 ml were distributed into 2 ml microcentrifuge tubes. Sterile, 0.3 ml glass conical inserts (glass inlets for HPLC; Supelco), containing 5 µl Na₂¹⁴C₃ (Amersham), corresponding to 3.7 × 10⁵ Bq (spec. act. 2.11 GBq mmol⁻¹), at the bottom, were placed into these microcentrifuge tubes. A 5 µl droplet of 20 % (v/v) H₂SO₄ was then placed on the wall of the glass inserts, close to the

top. The microfuge tube was closed and sealed with Parafilm. A short run in the microcentrifuge was used to mix the droplets of $\text{Na}_2^{14}\text{CO}_3$ and H_2SO_4 and to generate labelled CO_2 , without direct contact between the cells and the sodium carbonate. Incubation was performed in an Eppendorf thermomixer at 35 °C. Samples of the cell suspension were taken after 90 min, 3 h and 16 h incubation. Total radioactivity incorporated into washed, hot-trichloroacetic-acid-precipitable material was determined using scintillation cocktail and an LS 6500 scintillation counter (Beckman) (Amaro *et al.*, 1991).

Polar lipid fatty acid analysis. Lipids were extracted using a modified Bligh–Dyer procedure, as described previously (Bligh & Dyer, 1959; Vancanneyt *et al.*, 1996). All solvents were freshly distilled and all glassware used was rinsed with dichloromethane. Wet cells (0.2 g) were suspended in 100 ml methanol/dichloromethane/phosphate buffer (52.6:26.3:21.1), sonicated for 15 min (Labsonic U; Braun) and incubated overnight at room temperature. Additional methanol/dichloromethane/phosphate buffer (35.4:61:57) was added, followed by an additional 5 min ultrasonic treatment. The samples were centrifuged at 5860 g for 15 min to separate the phases. The dichloromethane phase was filtered through dry sodium sulfate and a hydrophobic filter. The methanol/phosphate buffer phase was reextracted by the addition of 25 ml dichloromethane, followed by centrifugation and filtration. This total lipid fraction was used for analysis by mass spectrometry. The total lipid fraction was reduced in volume by using a rotary evaporator and further fractionated by column chromatography (B&J inert SPE, Silica; Burdick & Jackson). The column was conditioned by overnight heating at 100 °C and, after cooling to room temperature, with 10 ml dichloromethane. The lipids were fractionated by sequential elution with dichloromethane, acetone and methanol, which resulted in three fractions of different polarity: neutral, glyco- and phospholipids. The eluates were collected and dried under nitrogen.

Fast-atom-bombardment mass spectrometry (FAB-MS). FAB-MS was performed, in the negative mode with a mixture of triethanolamine and tetramethylurea (2:1, v/v) as matrix, on the first of two mass spectrometers of a tandem high-resolution instrument of E1B1E2B2 configuration (JMS-HX/HX110A; JEOL) at 10 kV accelerating voltage. Resolution was set to 1:2000. The JEOL FAB gun was operated at 6 kV with xenon.

Tandem mass spectrometry. Negative daughter-ion spectra were recorded using all four sectors of the tandem mass spectrometer. High-energy collision-induced dissociation (CID) took place in the third field free region. Helium served as the collision gas at a pressure sufficient to reduce the precursor ion signal to 30% of the original value. The collision cell was operated at ground potential in the negative mode. Resolution of MS2 was set to 1:1000. FAB-CID spectra (linked scans of MS2 at constant B/E ratio) were recorded at 300 Hz filtering with a JEOL DA 7000 data system.

DNA G+C content. The G+C content of genomic DNA isolated from strain Y^T was determined directly by HPLC with a Nucleosil 100-5 C-18 column (Macherey–Nagel), according to methods described previously (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). Purified, non-methylated lambda phage DNA (Sigma) was used as a control.

16S rRNA gene sequence determination and analysis of phylogenetic relationships. Total DNA was isolated from

50 ml cells from a late-exponential phase culture by using the CTAB miniprep protocol for bacterial genomic DNA preparations (Wilson, 1987). 16S rRNA genes were amplified by PCR using the forward primer 16FpgA (5'-TCCGGTTGATCCTGCCGG-3') and the reverse primer 16RpgA (5'-TACGGYTACCTTGTTACGACTT-3'), corresponding to positions 3–20 of the 16S rRNA of *Haloferax volcanii* (Gupta *et al.*, 1983) and positions 1492–1513 of the 16S rRNA gene of *Escherichia coli* (Brosius *et al.*, 1981), respectively. Direct sequencing of the PCR-amplified DNA was carried out using an automated DNA sequencer and *Taq* cycle-sequencing reactions, according to the protocols of the manufacturer (Perkin-Elmer Applied Biosystems). Sequence data were compared initially with 16S rRNA gene sequences using the electronic mail servers at the Ribosomal Database Project (RDP; Maidak *et al.*, 1999) and FASTA version 3.0t71 (Pearson & Lipman, 1988) to search DNA sequence databases. Evolutionary distances and phylogenetic relationships were estimated using the programmes of the Phylogeny Inference Package (PHYLP version 3.57c) and dendrograms were derived using the additive tree model of the FITCH program (Fitch–Margolish and least-sequences distance methods) with random order input of sequence and the global rearrangement option (Felsenstein, 1989).

RESULTS

Morphology

Vegetative cells of strain Y^T appeared irregular and pleomorphic by transmission electron microscopy (Fig. 1). The irregular morphology of shadowed (Fig. 1d) and negatively stained (Fig. 1e, f) cells was observed to resemble that of cells of *Mycoplasma*. The cells ranged from 1.0 to 3.0 µm in length and from 0.3 to 1.0 µm in width. The cytoplasm appeared homogeneous and the chromosome was visualized as electron-translucent aggregates (Fig. 1b; asterisks). The cells did not exhibit a distinct cell wall, possessing only a cytoplasmic membrane as the peripheral barrier, 4.1–6.8 nm thick, covered with a thin layer of amorphous, electron-dense material (Fig. 1b, c). A characteristic feature of strain Y^T is the ability to form budding processes, which appeared tubular or vesicular in shape (Fig. 1a, d, f; filled arrows) and which tended to form septation annuli (Fig. 1e; open arrowheads). The tubular extrusions were observed to range from 85 to 142 nm in diameter and up to 1 µm in length. The process of budding, or formation of extrusions, is seen in Fig. 1(b), where the cell forms a tip (open double arrows) as the initiation and a vesicle, which nearly terminates its budding (filled double arrows). Different forms of 'offspring' are thereby released (Fig. 1d–f; indicated by an open arrow in Fig. 1d).

Relation to temperature and pH

At the optimal pH for growth of 1.7, strain Y^T grew within a temperature range of 15–45 °C, having an optimum at 35 °C (Fig. 2b). At the optimal temperature, growth occurred within the pH range 1.3–2.2 (Fig. 2c). Cells were observed by phase-contrast microscopy to be osmotic and pH-sensitive.

Fig. 1. Ultrastructure of vegetative cells. (a) Longitudinal ultrathin section showing a homogeneous cytoplasmic matrix; budding processes are indicated by arrows. (b) Detailed views of budding process as initial tip-formation (open double arrows) and almost-complete separation of vesicular offspring (filled double arrows); asterisks indicate the condensed bacterial chromosome. (c) High-magnification view of the cytoplasmic membrane, as indicated by opposing arrows. (d) Pt/C-shadow-cast bacterial cells. Open arrow indicates a tubular cellular offspring and filled arrows point to tubular extrusions; arrowhead gives shadowing direction. (e) Cell with bipolar budding processes; arrowheads indicate the septa. (f) Cellular extrusions of different sizes. Bars: 500 nm (a, d, e, f); 100 nm (b, c).

Fig. 2. Growth of *Ferroplasma acidiphilum* strain Y^T on modified 9K medium supplemented with FeSO₄. (a) Growth curve of strain Y^T growing under conditions of optimal pH (1.7) and temperature (35 °C). (b) Growth of isolate Y^T at various temperatures, pH 1.7. ●, Protein (mg l⁻¹); ■, amount of Fe²⁺ oxidized (mM); after 96 h growth. (c) Influence of pH on the growth of the isolate at 35 °C. (d) Effect of yeast extract and vitamin addition on biomass yield of a culture of strain Y^T at pH 1.7 and 35 °C. Protein levels (b–d) and Fe²⁺ concentrations (b) were measured after 96 h growth.

Oxidation of inorganic substrates

The growth curve of the isolate in the modified medium 9K at optimal pH and temperature is shown in Fig. 2(a). Growth of strain Y^T could be detected after 24–35 h, when the ferrous iron started to be oxidized and the medium turned yellow. After 50–55 h cultivation, the medium became red and, after 80–90 h, reddish-brown, due to oxidation of Fe²⁺. Typically, strain Y^T oxidized ferrous FeSO₄ and pyrite (Fe₂S). Sulfide minerals, such as sphalerite, galenite and antimonite, were not oxidized. Strain Y^T was capable of growing on MnSO₄, although it did not yield as much biomass as when grown in medium supplemented with Fe²⁺ (6.5 mg protein g⁻¹ Mn²⁺ and 12 mg protein g⁻¹ Fe²⁺). Neither elemental sulfur in crystalline or colloid form nor reduced compounds of sulfur, tetrathionate and thiosulfate, were oxidized.

Growth on organic substrates

Strain Y^T was not capable of growth on any of the organic substrates listed in Methods, although the addition of yeast extract was observed to be essential for growth (Fig. 2d). Growth of strain Y^T was strongly inhibited by the presence of yeast extract in amounts greater than 0.2% and growth was not detected on

yeast extract alone in the absence of Fe²⁺. A vitamin solution could be substituted for yeast extract, although the specific biomass yield after 96 h growth decreased from 12 to 5.5 mg protein g⁻¹ Fe²⁺.

Fixation of inorganic carbon

Strain Y^T was observed to incorporate inorganic carbon, added to the culture as ¹⁴CO₂. After 90 min, the radioactivity of incorporated ¹⁴C was 535 Bq mg⁻¹ protein. Exposure of the biomass to ¹⁴CO₂ for longer times, 3 and 16 h, did not increase the incorporated radioactivity significantly (700 and 725 Bq mg⁻¹ protein, respectively). One possible explanation for this is the limitation of growth due to a lack of ferrous iron, which, after a short period of growth, possibly within a few minutes, was almost completely depleted by the very high concentration of cells (residual concentrations of Fe²⁺ were 23, 5 and <0.1 mM after 90 min, 3 h and 16 h exposure).

Oxygen requirement

Strain Y^T was observed to grow strictly aerobically. No measurable growth occurred under anaerobic conditions in the presence of H₂/CO₂, CO₂ alone or with formate or acetate, with or without Fe²⁺.

Fig. 3. (—)FAB mass spectrum of the total lipid fraction of *Ferroplasma acidiphilum* strain Y^T. Archaetidyl glycerol gave the main ion at *m/z* 805, while the molecular ion of archaetidic acid is found at *m/z* 731. The ions of low intensity at *m/z* 1446 and 1608 were formed from the dimers of archaetidic acid and archaetidyl glycerol, respectively. The ion at *m/z* 507 is the result of the neutral loss of phytanol from archaetidyl glycerol.

Antibiotic sensitivity

Strain Y^T was resistant to ampicillin (50 µg ml⁻¹), which inhibits cell wall formation in bacteria, as well as to chloramphenicol (> 10 µg ml⁻¹), kanamycin (50 µg ml⁻¹), rifampicin (25 µg ml⁻¹) and streptomycin (50 µg ml⁻¹). Strain Y^T was sensitive to tetracycline (2 µg ml⁻¹) and gentamicin (2 µg ml⁻¹).

Analysis of cellular lipids

The phospholipids were purified by column chromatography or were determined in the extract of total lipids. The structures of the individual phospholipids present in strain Y^T were identified by MS. The main phospholipid was observed to be archaetidyl glycerol, although some archaetidic acid could also be detected (Fig. 3). Dimers of both ether lipids were observed in small amounts. No tetraethers were detected in the total lipid extract of strain Y^T.

DNA G + C content

The G + C content of the genomic DNA was determined to be 36.5 mol %.

Molecular phylogenetic analysis

The 16S rRNA gene sequence of strain Y^T was observed to cluster with those of organisms representing the order 'Thermoplasmatales' within the *Euryarchaeota* (Fig. 4). Strain Y^T exhibited a relatively deep branching, with uncertainty concerning its closest phylogenetic affiliation, although, according to the method of analysis used, strain Y^T was estimated to be phylogenetically most closely related to species of the genera *Picrophilus* and *Thermoplasma*, albeit with only 89.9 and 87.7% 16S rRNA sequence identity, respectively, to *Picrophilus oshimae* and *Thermoplasma acidophilum*.

DISCUSSION

The order 'Thermoplasmatales' or 'Thermoplasmatales' *sensu* Woese (Woese, 1987; Woese *et al.*, 1990; Segerer & Stetter, 1992b; Schleper *et al.*, 1996) is represented by the facultatively anaerobic genus *Thermoplasma* (Darland *et al.*, 1970; Segerer *et al.*, 1988; Segerer & Stetter, 1992b) and the strictly aerobic genus *Picrophilus* (Schleper *et al.*, 1995, 1996), the species of which are thermoacidophilic, heterotrophic organisms. Phylogenetically, these organisms are clustered within one of the two main branches of the domain *Archaea*, the *Euryarchaeota*, which also includes methanogenic and halophilic archaea (Woese *et al.*, 1990). On the basis of 16S rRNA sequence comparisons, strain Y^T, isolated from gold-containing pyrite ore concentrate, occupies a distinct position between the genera *Picrophilus* and *Thermoplasma*.

Other characteristic features of the 'Thermoplasmatales' are the lack of a distinct cell wall in representatives of the genus *Thermoplasma* and the presence of an S-layer in species of *Picrophilus* and a specific morphology: cells of species of *Thermoplasma* have variously sized, filamentous, coccoid-, disc- and club-shaped forms, that can be observed in the same culture, whereas cells of the *Picrophilaceae* are irregular cocci (Segerer & Stetter, 1992b; Schleper *et al.*, 1995, 1996). The lack of a cell wall also confers a high osmotic and pH sensitivity upon strain Y^T, as well as insensitivity to ampicillin, as is the case for the 'Thermoplasmatales'.

The principal phenotypic characteristics of isolate Y^T, apart from its acidic origin, lack of a cell wall and low G + C content, do not agree, however, with those of other species belonging to the order 'Thermoplasmatales' (Table 1). The most important difference from other members of the 'Thermoplasmatales' is the obligate autotrophy of strain Y^T, which is the only organism of this phylogenetic branch reported to date to fix inorganic carbon. In that strain Y^T assimilates CO₂ and obtains energy at the expense of the oxidation of Fe²⁺ and Mn²⁺, it is metabolically similar to some representatives of the order *Sulfolobales*, e.g. *S. metallicus* and *A. ambivalens*, the only strict chemolitho-autotrophs of the order, as well as to *A. brierleyi*, *Metallosphaera sedula* and *Metallosphaera prunae*,

Fig. 4. The estimated phylogenetic position of *Ferroplasma acidiphilum* strain Y^T (= DSM 12658^T), derived from 16S rRNA gene sequence comparisons, among the major evolutionary lineages of the Archaea. The sequence data for other organisms were obtained from the GenBank/EMBL databases under the following accession numbers: *Archaeoglobus fulgidus*, X05567; *Desulfurococcus mobilis*, M36474; *Halobacterium halobium*, AJ002949; *Methanobacterium formicicum*, M36508; *Methanomicrobium mobile*, M59142; *Picrophilus oshimae*, X84901; *Pyrodictium occultum*, M21087; *Sulfolobus acidocaldarius*, D14053; *Thermococcus celer*, M21529; *Thermoplasma acidophilum*, M32298; *Thermoproteus tenax*, M35966; *Escherichia coli*, J01695. The scale bar represents 10 substitutions per 100 nucleotide positions.

Table 1. Comparison of key characteristics of the archaea belonging to the order 'Thermoplasmales'

Data were taken from Schleper *et al.* (1995) (*Picrophilus*) and Darland *et al.* (1970), Segerer *et al.* (1988) and Segerer & Stetter (1992b) (*Thermoplasma*). +, Positive reaction or growth; –, negative reaction or growth.

Characteristic	<i>Picrophilus</i> spp.	<i>Thermoplasma</i> spp.	<i>Ferroplasma acidiphilum</i>
Morphology	Irregular cocci	Pleomorphic	Pleomorphic
Flagella	+	+	–
Autotrophy	–	–	+
Fe ²⁺ oxidation	–	–	+
Aerobic growth	+	+	+
Anaerobic growth	–	+	–
Temperature for growth (°C):			
Optimum	60	60	35
Range	45–65	33–67	15–45
pH for growth:			
Optimum	0.7	1–2	1.7
Range	0.1–3.5	1–4	1.3–2.2
S-layer	+	–	–
DNA G+C content (mol%)	36	46	36.5

which, however, are also able to use a number of organic compounds as sole carbon and energy sources (Seeger *et al.*, 1986; Huber *et al.*, 1989; Huber & Stetter, 1991; Fuchs *et al.*, 1995, 1996). In contrast to the *Sulfolobales*, isolate Y^T does not use elemental sulfur or its reduced forms.

Apart from the nutritional requirements, isolate Y^T exhibits a marked difference from the 'Thermoplasmatales' in the composition of its cellular lipids. Archaeidic acid and archaeidyl glycerol, which comprise the majority of the total lipids of the organism, are commonly detected in organisms of the halophilic and methanogenic lineages of the *Euryarchaeota* branch of the *Archaea*. Archaeidic acid was reported from *Halobacterium cutirubrum* and other halophilic archaea (Fredrickson *et al.*, 1989; Lanzotti *et al.*, 1989) and from *Methanobacterium thermoautotrophicum* (Nishihara & Koga, 1990). Archaeidyl glycerol has been reported from species of *Halobacterium* (Kushwaha *et al.*, 1982), *Methanosarcina barkeri* (Nishihara & Koga, 1995), *Methanospirillum hungatei* (Kushwaha *et al.*, 1981) and an unidentified, extremely halophilic archaeon (Upasani *et al.*, 1994). Lipids characteristic for the genera *Thermoplasma* and *Picrophilus*, i.e. di-isopropanol 2,3-glycotetraether and bis-phytanyltetraethers (Langworthy, 1985; Schleper *et al.*, 1995), were not found in strain Y^T.

A significant feature that distinguished strain Y^T from other members of the 'Thermoplasmatales', with which it clusters phylogenetically, and from the *Sulfolobales*, with representatives of which it shares a number of physiological traits, is the range of temperatures at which growth is observed. Strain Y^T has an optimum temperature for growth of 35 °C and a maximum of 45 °C, whereas the minimum temperature for growth of *A. brierleyi* and *P. oshimae* is 45 °C, and other thermoacidophilic archaea of the 'Thermoplasmatales' and *Sulfolobales* exhibit minimum temperatures for growth that are 10–15 °C higher (Seeger & Stetter, 1992a, b; Schleper *et al.*, 1995). Thus, the first part of the descriptive adjective thermoacidophilic is not applicable to isolate Y^T, which conforms to the ranks of mesophilic prokaryotes and is, by this criterion, similar to the autotrophic species of the bacterial genera *Thiobacillus* and 'Leptospirillum', which also oxidize ferrous iron chemolithoautotrophically (Temple & Colmer, 1951; Markosyan, 1972).

A recent 16S rRNA gene sequence analysis of a total DNA extract from a stable microbial consortium of a copper-leaching reactor reported the detection of an archaeon that probably represented a novel family within the 'Thermoplasmatales' (Vásquez *et al.*, 1999). The archaeon, morphologically similar to species of *Thermoplasma*, exhibited only two nucleotide differences from the 16S rRNA gene sequence of strain Y^T over 912 homologous nucleotide positions. The authors, however, failed to obtain a pure culture of the organism, which was presumed to be dependent on associated autotrophic bacteria such as *Thiobacillus*

ferrooxidans, *Thiobacillus thiooxidans*, 'Leptospirillum ferrooxidans', heterotrophic *Acidophilium* species and heterotrophic fungi.

It is apparent from its phenotypic properties and the differences in 16S rRNA gene sequences that the iron-oxidizing, acidophilic archaeon, strain Y^T, isolated from an arsenopyrite ore bioleaching reactor, cannot be assigned to any previously recognized genus and represents a phylogenetic lineage that corresponds to a new species in a new genus, within a new family, under the epithet *Ferroplasma acidiphilum* fam. nov., gen. nov., sp. nov.

Description of *Ferroplasmaceae* Golyshina *et al.* fam. nov.

Ferroplasmaceae (Fer.ro.plas.ma'ce.ae. M.L. *ferro* pertaining to ferrous iron; Gr. neut. n. *plasma* something shaped or moulded; L. *-aceae* ending denoting a family; M.L. *Ferroplasmaceae* a family of ferrous-iron-oxidizing forms).

A family belonging to the order 'Thermoplasmatales', separate and distinct from the 'Thermoplasmaceae' and the *Picrophilaceae*, which contains cell-wall- and S-layer-lacking, ferrous-iron-oxidizing, chemolithoautotrophic, acidophilic organisms. The segregation of these organisms into a new family is justified (i) by their distinct phylogenetic position (the 16S rRNA sequence is nearly equally distant, i.e. >10% difference, from representatives of existing families, *Picrophilus oshimae* and *Thermoplasma acidiphilum*), (ii) by obligate chemolithoautotrophy, whereas other members of the 'Thermoplasmatales', the 'Thermoplasmaceae' and the *Picrophilaceae*, are obligate heterotrophs that are not able to grow autotrophically, (iii) by their mesophilic growth temperature range and (iv) by the dominance of archaeidic acid and archaeidyl glycerol as membrane lipids and the complete absence of tetraether lipids, which are predominant in the 'Thermoplasmaceae' and the *Picrophilaceae*.

Description of *Ferroplasma* Golyshina *et al.* gen. nov.

Ferroplasma (Fer.ro.plas'ma. M.L. *ferro* pertaining to ferrous iron; Gr. neut. n. *plasma* something shaped or moulded; M.L. *Ferroplasma* a ferrous-iron-oxidizing form).

Cells are irregular cocci, varying from spherical to filamentous, forming duplex and triplex forms. Gram-negative. Strict aerobes. Cell wall and S-layer are absent. Acidophilic. Strictly chemolithoautotrophic; no organic compounds have been found that are used as carbon sources. Oxidizes Fe²⁺ from FeSO₄ and pyrite (Fe₂S); oxidizes Mn²⁺ from MnSO₄. Mesophilic. Principal lipids are archaeidic acid and archaeidyl glycerol. The type and only species of the genus is *Ferroplasma acidiphilum*.

Description of *Ferroplasma acidiphilum* Golyshina et al. sp. nov.

Ferroplasma acidiphilum (a.ci.di'phi.lum. M.L. neut. n. *acidum* an acid; Gr. adj. *philos* loving; M.L. neut. adj. *acidiphilum* acid-loving).

Morphology and nutritional requirements are as described for the genus. G+C content of DNA is 36.5 mol%. Growth occurs between temperatures of 20 and 45 °C with an optimum at 35 °C and at pH 1.3–2.2 with an optimum at pH 1.7.

Strain Y^T is the only and type strain of *Ferroplasma acidiphilum*. *Ferroplasma acidiphilum* strain Y^T has been deposited in the DSMZ as strain DSM 12658^T.

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