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Genome-based classification of *Acidihalobacter prosperus* F5 (=DSM 105917=JCM 32255) as *Acidihalobacter yilgarnensis* sp. nov.

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Abstract

The genus *Acidihalobacter* has three validated species, *Acidihalobacter ferrooxydans*, *Acidihalobacter prosperus* and *Acidihalobacter aeolinanus*, all of which were isolated from Vulcano island, Italy. They are obligately chemolithotrophic, aerobic, acidophilic and halophilic in nature and use either ferrous iron or reduced sulphur as electron donors. Recently, a novel strain was isolated from an acidic, saline drain in the Yilgarn region of Western Australia. Strain F5^T has an absolute requirement for sodium chloride (>5 mM) and is osmophilic, growing in elevated concentrations (>1 M) of magnesium sulphate. A defining feature of its physiology is its ability to catalyse the oxidative dissolution of the most abundant copper mineral, chalcopyrite, suggesting a potential role in biomining. Originally categorized as a strain of *A. prosperus*, 16S rRNA gene phylogeny and multiprotein phylogenies derived from clusters of orthologous proteins (COGS) of ribosomal protein families and universal protein families unambiguously demonstrate that strain F5^T forms a well-supported separate branch as a sister clade to *A. prosperus* and is clearly distinguishable from *A. ferrooxydans* DSM 14175^T and *A. aeolinanus* DSM14174^T. Results of comparisons between strain F5^T and the other *Acidihalobacter* species, using genome-based average nucleotide identity, average amino acid identity, correlation indices of tetra-nucleotide signatures (Tetra) and genome-to-genome distance (digital DNA–DNA hybridization), support the contention that strain F5^T represents a novel species of the genus *Acidihalobacter*. It is proposed that strain F5^T should be formally reclassified as *Acidihalobacter yilgarnenesis* F5^T (=DSM 105917^T=JCM 32255^T).

INTRODUCTION

Bioleaching is a technique where acidophilic micro-organisms are used to catalyse the extraction of metals from mineral ores through the oxidation of metal sulfides, a technology referred to to generically as biomining [1]. As many accessible higher-grade metal ore bodies are now depleted, lower-grade ores are being increasingly exploited, and bioleaching can have both economic and environmental benefits for processing these materials [2]. However, biomining has long faced the challenge of the negative effect of the presence of salt (sodium chloride) in ores and process waters. The ability of bioleaching micro-organisms to tolerate salt varies between genus and species, but most bioleaching micro-organisms cannot tolerate the levels of chloride present in seawater and can be inhibited by concentrations as low as 6.6 g l^{-1} [3–5]. However, the presence of salt has been shown to enhance the abiotic leaching of the recalcitrant but also most abundant copper-containing mineral in the lithosphere, chalcopyrite (CuFeS$_2$). Therefore, the use of halophilic micro-organisms that are also

capable of tolerating low pH while being able to catalyse the oxidative dissolution of chalcopyrite would be of major benefit to the biomining industry [4, 5]. Furthermore, as freshwater resources become increasingly scarce, the mining industry would benefit from using seawater at mining sites to reduce the costs associated with desalination plants [2].

Due to the limited environments that are available for the discovery of the unique micro-organisms that inhabit low pH and highly saline environments and have the ability to oxidize metal sulfide minerals, it is important to isolate and characterize these prokaryotes [5].

The genus *Acidihalobacter* represents one such group of ferrous iron- and sulfur-oxidizing bacteria that are both extremely acidophilic and halotolerant (tolerating up to 1283 mM NaCl) [6–8]. Three members of the genus *Acidihalobacter* have been isolated from the Vulcano region Italy. *Acidihalobacter prosperus* DSM 5130^T (previously '*Thiobacillus prosperus*') was isolated from a geothermally heated seafloor at Porto di Levante while the type strains of *Acidihalobacter aeolinanus* (previously '*Acidihalobacter prosperus* DSM 14174') and *Acidihalobacter ferrooxydans* (previously '*Acidihalobacter ferrooxidans* DSM 14175') were isolated from a shallow acidic pool by the shore of Baia de Levant [9, 10]. All are members of the family *Ectothiorhodospiraceae* of the class *Gammaproteobacteria* in the genus *Acidihalobacter*, and each has been characterized as the type strain of their respective species [11–14].

More recently, a novel bacterial strain, designated as F5^T and belonging to genus *Acidihalobacter*, was isolated from an acidic saline drain in the Yilgarn region of Western Australia [5]. The isolate was initially considered to be a strain of *A. prosperus* due to the high sequence similarity (98.7%) of its 16S rRNA gene to the latter [8]. However, a defining feature of this strain that distinguishes it from other strains of *Acidihalobater* was its ability to leach chalcopyrite at 508 mM NaCl. This makes it a potentially valuable isolate for the industrial biorecovery of copper through saline water bioleaching [8].

The genome of strain F5^T is the only available complete genome of a halotolerant acidophile to date, as well as the first complete genome for a member of the genus *Acidihalobacter* [8]. The completeness of its genome provides an opportunity for studies of its metabolic capabilities as well as clarification of its taxonomy. Genome-based classification of the other members of the genus *Acidihalobacter* has recently been completed and has proven to provide a more robust approach for the re-evaluation of taxonomy using bioinformatics-based phylogenomic strategies that are more accurate than 16S rRNA gene phylogeny and morphology alone [14, 15].

METHODS

Isolation of strain F5^T

Strain F5^T was isolated from an enrichment culture obtained from an acidic saline drain in the Yilgarn region in Western Australia (pH 2.1, 463 mM chloride, 25 mM iron (II); GPS coordinates −31.070302° S, 117.43901° E) [5, 8]. The enrichment culture was inoculated onto overlay plates [16] (0.625% agarose pH 2.5) enriched with (i) FeSO$_4$, (ii) K$_2$S$_4$O$_6$ or (iii) a mixture of both, containing 214 mM NaCl. Single colonies picked from the solid media were resuspended in liquid media containing either 50 mM ferrous sulphate or 5 mM potassium tetrathionate, basal salts (3 mM (NH$_4$)$_2$SO$_4$, 1.6 mM MgSO$_4$ and 2.9 mM KH$_2$PO$_4$) and trace elements (pH 1.8) [7]. DNA extraction and 16S rRNA gene sequences were obtained through Sanger sequencing as described previously [17].

Tolerance to temperature, pH and NaCl

A pure culture of strain F5^T was maintained at 30 °C in basal salts containing 50 mM ferrous sulphate and 5 mM potassium tetrathionate at pH 2.5 as described above, and DNA was extracted from these cultures for genome sequencing as described previously [8]. Growth of the isolate was tested at a range of temperatures (17–42.5 °C), pH levels (pH 1– 5) and sodium chloride concentrations (0–1.71 M; data not shown). Bioleaching studies were performed as described elsewhere [8].

For the purpose of this study, further growth tests were performed on various liquid and solid media, including the growth of strain F5^T on elemental S, H$_2$ and in K$_2$S$_4$O$_6$-free media containing 10 mM Fe(II) and 200 mM MgSO$_4$. Aerobic growth was tested at different concentrations of MgSO$_4$ (0, 50, 100, 200, 500 and 1000 mM). When the cultures failed to grow, 25 mM NaCl was added in the medium and incubated further for up to 24 days. Tests for optimum concentration of NaCl in cultures containing 200 mM MgSO$_4$ were then performed using 0, 5, 10, 25 and 50 mM NaCl.

Electron microscopy

Electron microscopic studies of strain F5^T were performed using the method described previously for the type strain of *A. prosperus* [18].

Selection of members for phylogenetic assignment

Members for inclusion in the study were identified from the 30 closest phylogenetic neighbours as given by *ab initio* comparisons of GLIMMER3 gene candidates with a set of universal proteins and up to 200 unduplicated proteins in the SEED and Rapid Annotation of Microbial genomes using Subsystems Technology (RAST) [19, 20]. These were verified by comparison to the sequences previously used for the reclassification of the type strain of *A. prosperus* [15], as well as by comparison with nucleotide databases after running a BLASTn-based script using an E-value threshold of 1e-5 and the databases GREENGENES, RDP and SILVA [21–23].

A total of 15 genomes, including the four members of the genus *Acidihalobacter*, were selected for inclusion into the following phylogenetic tree reconstructions. *Halothiobacillus neapolitanus* ATCC 23641 was used as an outgroup (Table S1, available in the online version of this article).

Closest phylogenetic neighbours of the genus *Acidihalobacter* were selected based on *ab initio* comparisons of GLIMMER3 and RAST [19, 20]. A total of 14 organisms of the order *Chromatiales*, including the three validated members of the genus *Acidihalobacter* together with strain F5[T], were selected for inclusion in phylogenetic tree reconstructions.

PHYLOGENETIC TREE RECONSTRUCTION

16S rRNA gene phylogeny

16S rRNA genes of *Acidihalobacter* species were identified by comparison of genomic sequences against 16S rRNA databases GREENGENES [21], RDP [22] and SILVA [23] by BLASTn [24] using an E-value threshold of 1e-5. Sequences of the taxonomically related genomes from the order *Chromatiales* were selected from NCBI databases to be included in the 16S rRNA gene phylogenetic tree. All 16S rRNA gene sequences were aligned in MAFFT version 7 with the L-INS-i iterative refinement [25, 26]. The phylogenetic tree was reconstructed with IQTREE, using 1000 replications as bootstrap support [27, 28] with best model fit by IQTREE (TN+F+I+G according to the Bayesian information criterion) [29].

Multi-locus sequence analysis (MLSA)

A set of 30 ribosomal proteins associated with COG markers (Table S2) were obtained from the DOE Joint Genome Institute – Integrated Microbial Genomes and Microbiome Samples website (https://img.jgi.doe.gov/cgi-bin/m/main.cgi) for each micro-organism in the study [30, 31]. A multi-locus phylogenomic tree was reconstructed by aligning a concatenated set of the 30 COGs sequences with L-INS-i iterative refinement in MAFFT version 7 and removal of unreliable regions with GBLOCKS [32, 33]. A maximum-likelihood tree with 1000 replicates was reconstructed with best-fit model LG+F+I+G according to the Bayesian information criterion using IQTREE [27, 28].

Nine conserved housekeeping genes (*argS*, *dnaQ*, *dnaN*, *era*, *gltA*, *gyrB*, *ppnK*, *rpoB* and *rpoD* [34–36]) were used to build a multi-gene species tree using a concatenated alignment from members of the order *Chromatiales* as described previously [14]. The contatenated alignment was reconstructed using the L-INS-i iterative refinement in MAFFT version 7 [25, 26], which were masked to remove unreliable regions with GBLOCKS [32, 33]. The maximum-likelihood tree was reconstructed with IQTREE using the bootstrap method with 1000 replicates [37] and the best-suited substitution model GTR+F+I+G selected by IQTREE .

Sequence-based methods for species circumscription

Calculation of average nucleotide identity was based on BLAST (ANIb) [24, 38, 39] and the correlation indexes of tetra-nucleotide signatures (Tetra) were conducted using Jspecies [39] and JspeciesWS (http://jspecies.ribohost.com/jspeciesws/#Analyse) [40]. The Genome-to-Genome Distance Calculator (GGDC) web tool (http://ggdc.dsmz.de/distcalc2.php) was used to calculate the digital DNA–DNA hybridization (dDDH) values [41, 42]. Average amino acid identity (AAI) [43] values were calculated with the CompareM tool (https://github.com/dparks1134/CompareM).

Gene prediction

Genes potentially encoding terminal oxidases and those involved in ferrous iron and reduced sulphur oxidation were predicted using a bidirectional BLASTP of the NR databases as described previously [14] and were visualized using Artemis [44].

RESULTS AND DISCUSSION

The genomes of the different *Acidihalobacter* isolates included in this study were previously obtained from pure cultures grown in acidified basal salts/trace elements medium supplemented with soluble iron and sulphur sources, and sodium chloride [8, 11–13]. However, key differences can be seen in the pH, temperature and optimum NaCl concentrations required for growth on soluble iron and sulphur sources as well as on the mineral sulfide ore pyrite (Table 1). While the type strain of *A. prosperus* has been shown to grow on sphalerite, chalcopyrite, arsenopyrite and galena as well as on H_2S, no leaching data is available for growth on these substrates [9]. Meanwhile, the type strains of *A. aeolianus* and *A. ferrooxydans* have previously been shown to oxidize chalcopyrite when in mixed culture; however, growth of pure isolates has not been tested [45, 46]. Furthermore, growth of the type strains of *A. aeolianus* and *A. ferrooxydans* is yet to be tested on other mineral ores. Strain F5[T] is the only isolate that has been shown to successfully leach the mineral ore pentlandite (at up to 1283 mM NaCl at pH 2.5 [8]). More importantly, it the only known isolate to leach the recalcitrant mineral chalcopyrite at up to 513 mM NaCl (pH 2.5), thereby suggesting its suitability to leach base metals from different sulfide ores at chloride ion concentrations of sea water or above (564 mM NaCl [6]).

Growth characteristics of strain F5[T]

The growth tests on strain F5[T] performed in this study showed that it can grow on both elemental sulphur and the reduced sulphur oxy-anion, tetrathionate. Growth was also observed when Fe(II) was provided as the sole electron donor and 200 mM $MgSO_4$ as the osmolyte, though no growth was seen when hydrogen was provided as the sole electron donor. Furthermore, the results of the tests using 0, 50, 100, 200, 500 and 1000 mM $MgSO_4$ as the osmotic stressor showed that no growth in the absence of salt. The addition of 25 mM NaCl resulted in good oxidation for cultures containing 50 and 500 mM but not 0 or 1 M $MgSO_4$. This shows that strain F5[T] has an absolute requirement for NaCl, as has previously been shown for the other members of the genus *Acidihalobacter*. When NaCl was added in increments (0, 5, 10, 25 and 50 mM) to cultures containing 200 mM $MgSO_4$ iron oxidation was evident in the cultures containing 10 and 25 mM NaCl within 3 days. After 4 days, some oxidation was seen in the 5 mM NaCl containing cultures, while after 12 days the 50 mM NaCl

Table 1. Comparison of genomic and phenotypic features of the four members of the genus *Acidihalobacter*

Feature	Genome			
	Acidihalobacter strain F5[T]	*Acidihalobacter prosperus* DSM5130[T]	*Acidihalobacter aeolianus* DSM 14174[T]	*Acidihalobacter ferrooxydans* DSM 14175[T]
Genome size (Mbp)	3.57	3.36	3.36	3.45
G+C content (mol%)	59.9	64.5	62.2	61.6
Predicted coding DNA sequence (CDS)	3233	3088	3194	3089
Plasmid	–	–	162484 bp (pABPV6)	–
tRNA genes	47	48	46	45
Sulphur oxygenase reductase (EC 1.13.11.55)	–	+	–	+
Temperature range for growth (°C)	24–33	20–45 [9]	26–42 [60]	26–43 [60]
Optimum temperature for growth (°C)	30	33 [9]	36 [60]	36 [60]
pH range for growth	2.0–4.0	1.0–4.5 [9]	1.5–3.0 [14]	1.0–3.0 [14]
Optimum pH for growth	2.5	2.0 [9]	1.8 [14]	1.8 [14]
NaCl range for growth (mM)	5–1283	70–1030 [18]	60–1283 [7]	60–856 [7]
Optimum NaCl (mM) for growth on $FeSO_4$ and $K_2S_4O_6$	428	340 [9]	428 [7, 45, 60]	428 [7, 60]
Optimum NaCl (mM) for growth on pyrite	513	N/A	256 [7]	856 [7]
Optimum NaCl (mM) for growth on chalcopyrite	254	NA	NA	NA

+, Present; –, absent; NA, not available.

cultures were well oxidized. The salt-free cultures showed very little oxidation even after 12 days. While $MgSO_4$ can meet its requirement for a relatively high external osmotic potential, a minimum of 5 mM NaCl is required for iron oxidation, with 10–25 mM being the optimum NaCl requirement in the presence of 200 mM $MgSO_4$. This NaCl requirement is lower than has been previously shown for the type strains of the three validated *Acidihalobacter* species (≥60 mM), although these values were determined with NaCl acting as the only significant osmolyte. In total, the results of the growth studies and absolute requirement of strain F5[T] for NaCl, confirms its obligately osmophilic nature.

Microscopy

Electron microscopic studies revealed that cells of strain F5[T] were 1–2 μm long straight rods (Fig. 1). Endospores were not detected.

Genome and gene information

Members of the family *Ectothiorhodospiraceae* are known to have a DNA G+C content within the range 50.5–69.7 mol% [47]. The bioinformatically inferred G+C content for the genome of strain F5[T] was previously found to be 59.9 mol%, which is lower than that of the other members in the genus, but

is still within the range of the family *Ectothiorhodospiraceae*. The genome of strain F5[T] is 3.57 Mbp and is predicted to have 3233 coding sequences with 47 tRNA genes [8]. Bioinformatically predicted terminal oxidases from the genomes
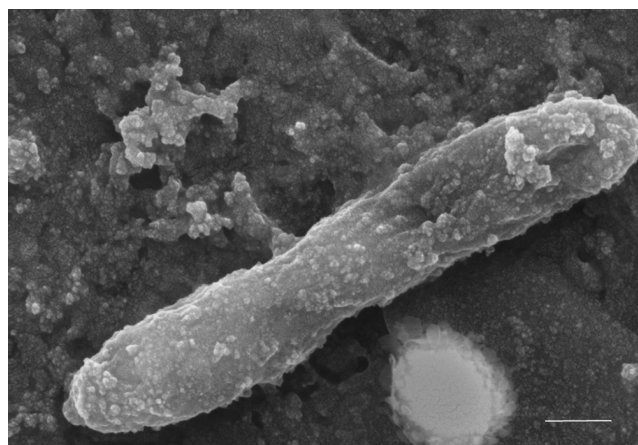
Fig. 1. Electron microscopy image of strain F5[T] grown in the presence of 214 mM NaCl. The scale bar is 200 nm.
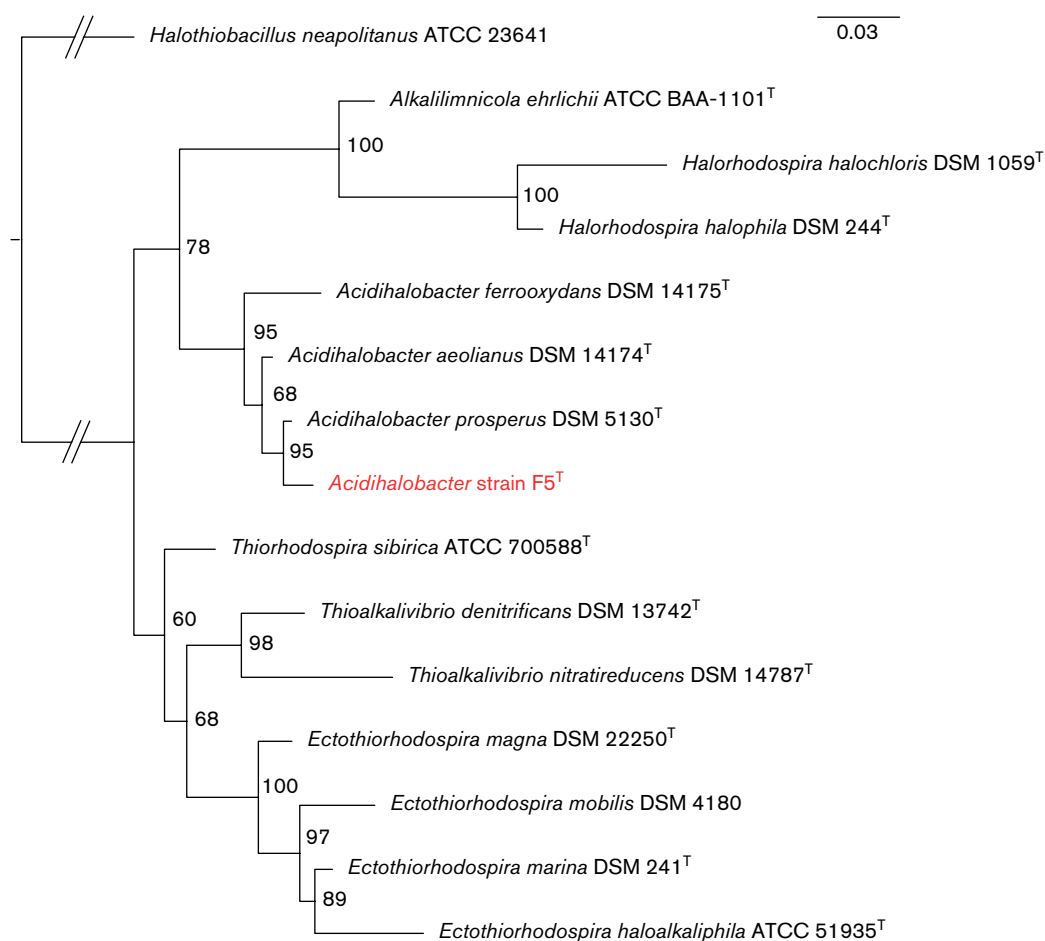
Fig. 2. Maximum-likelihood phylogenetic tree of 16S rRNA gene sequences of strain F5[T] (in red) and other phylogenetic relatives as described in the text. Bootstrap percentages (1000 replicates) are labelled at the nodes. Scale bar represents 0.03 nucleotide substitution per site. The genetic distance of *Halothiobacillus neapolitanus* ATCC 23641 is not to scale as indicated by the break lines //. The full list of NCBI accession numbers is given in Table S1.

of F5[T] were as for DSM14174[T] and DSM14175[T] and included aa_3 (EC 1.9.3.1), bo_3 (EC 1.10.3.10), bd-I (EC 1.10.3.14) and fumarate reductase (quinol, EC 1.3.5.1–1.3.5.4). Respiratory quinones predicted from the genomes include ubiquinone $ubiABDEGIHJX$ (EC 1.14.13.-, 1.14.12.240, 2.1.1.222, 2.1.1.64, 2.1.1.163, 2.1.1.201, 2.5.1.39, 2.5.1.129, 4.1.1.98). Phenotypic and genomic features of the four species of the genus *Acidihalobacter* are compared in Table 1. The genome is predicted to encode a rusticyanin gene cluster thought to be involved in Fe^{2+} oxidation [8]. The accession number of the genome sequence of strain F5[T] is CP017415.1.

Phylogeny based on 16S rRNA gene sequence analysis

A 16S rRNA gene phylogenetic tree of strain F5[T] and three validated members of the genus *Acidihalobacter* was reconstructed using ten validated species belonging to the family *Ectothiorhodospiraceae* of the order Chromatiales of the class Gammaproteobacteria using *Halothiobacillus neapolitanus* ATCC 23641 as an outgroup (Fig. 2). The tree agrees with

a previously published 16S rRNA gene phylogenetic tree in the placement of strain F5[T] within the genus *Acidihalobacter* and confirms its taxonomic position within the family *Ectothiorhodospiraceae* [8]. Strain F5[T] forms a separate branch as a sister clade to *A. prosperus* DSM 5130[T] that is well-supported (95% bootstrap support) and is clearly distinguishable from *A. ferrooxydans* DSM 14175[T] and *A. aeolinanus* DSM 14174[T].

Phylogeny based on multiple locus sequence analyses (MLSA)

Additional approaches were used to evaluate the phylogenomic position of strain F5[T]. Phylogenomic trees were reconstructed based on the sequences of 30 concatenated conserved ribosomal proteins [30, 31] (Fig. 3a) and nine concatenated housekeeping genes (Fig. 3b). These multi-locus sequence alignments were sufficiently long to allow mapping of their phylogenetic relationships [30, 34, 35]. Both trees consistently place strain F5[T] as a sister clade to *A. prosperus* DSM 5130[T] with 100% bootstrap support and clearly show that strain F5[T] forms a distinct branch from *A. ferrooxydans*

(a)

(b)
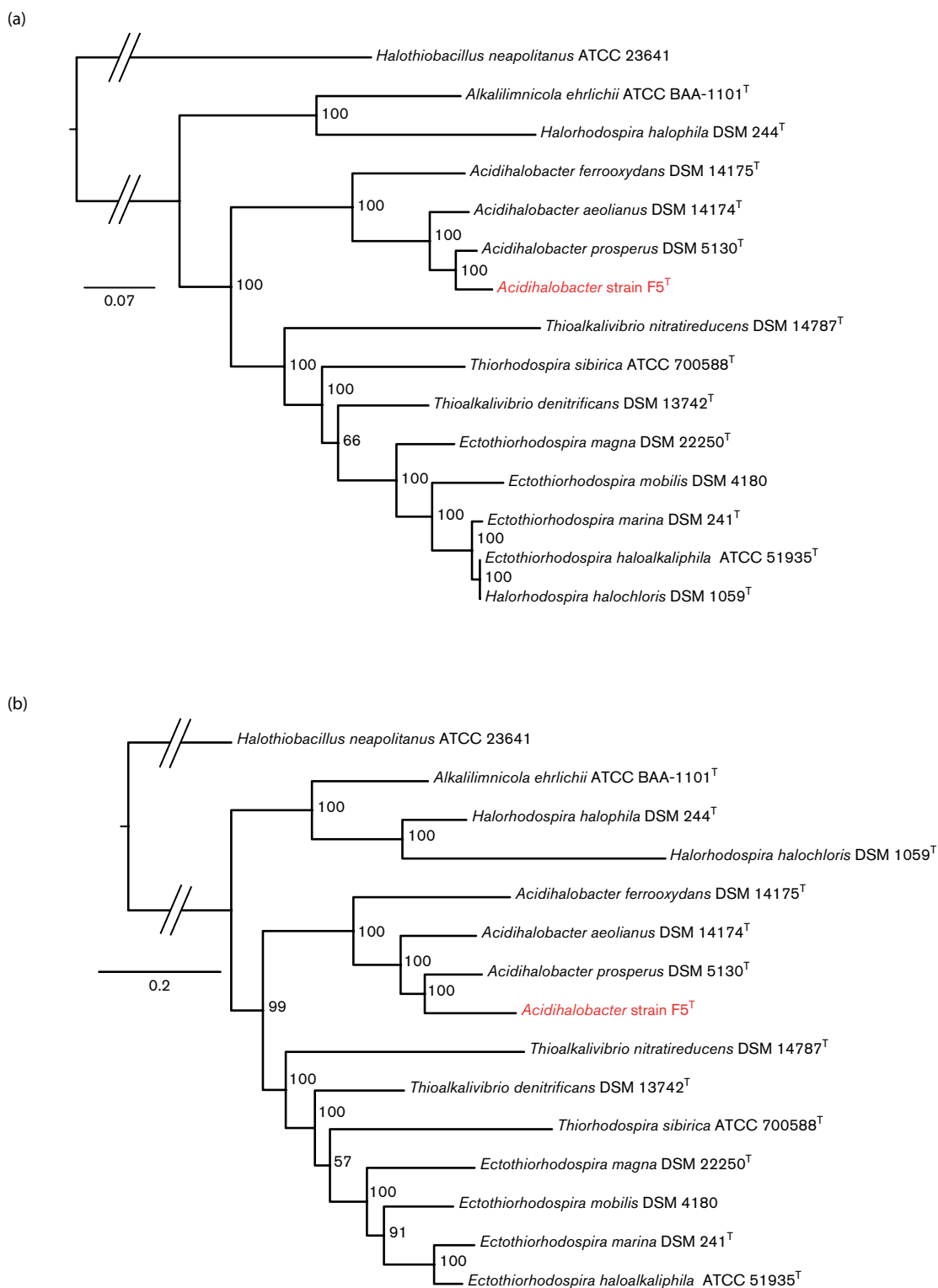
Fig. 3. Phylogenomic trees of 14 members of the order *Chromatiales* and *Halothiobacillus neapolitanus* ATCC 23641 as outgroup, including strain F5ᵀ (in red), based on (a) 30 concatenated conserved proteins from proposed 34 ribosomal proteins [30, 31] and (b) nine concatenated housekeeping genes. Statistically supported bootstrap values as percentages of 1000 replicates are labelled at the nodes. Scale bar represents 0.07 amino acid and 0.2 nucleotide changes per site, respectively. The full list of COG families is given in Table S2.
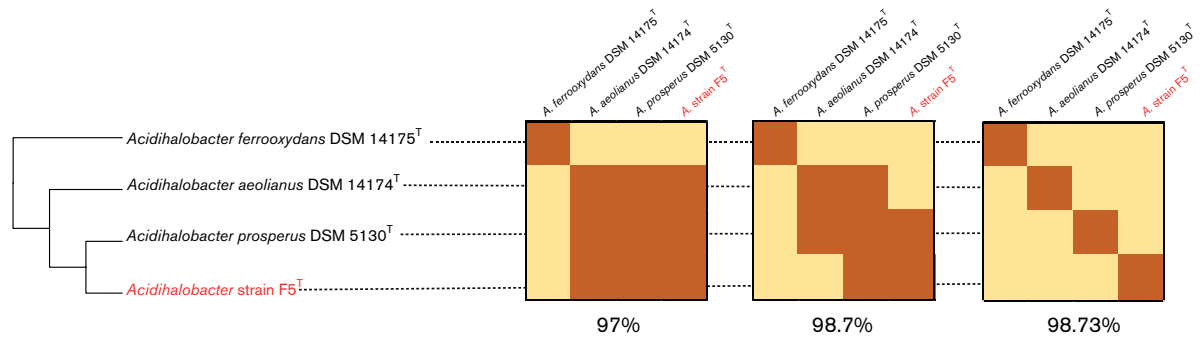
Fig. 4. Heat maps of the percent difference of 16S rRNA gene sequences between the three validated *Acidihalobacter* species and strain F5[T]. The results are displayed as a cladogram based on the 16S rRNA gene phylogenetic tree shown in Fig. 2, using 97, 98.7 and 98.73% 16S rRNA gene sequence similarity cutoff values (left to right, respectively).

DSM 14175[T] and *A. aeolianus* DSM 14174[T] agreeing with the 16S rRNA gene phylogenetic tree.

MLSA is a powerful tool for determining phylogenetic relationships but it is not widely used to discriminate species and subspecies because it is difficult to decide the depth of clustering that should be used as a threshold for differentiation [48].

### Phylogenetic distance based on percentage similarity of 16S rRNA gene sequences

16S rRNA gene sequence similarity analysis is frequently used to infer phylogenetic distance and is used in microbial classification and species identification [49]. The similarity of the 16S rRNA gene sequence of strain F5[T] to the three validated *Acidihalobacter* species is reported as a heat map comparison (Fig. 4).

Strain F5[T] is located in a sister clade to *A. prosperus* DSM 5130[T] but can be distinguished from it at a cutoff of 98.7% sequence similarity (Fig. 4). A cutoff of 97% 16S rRNA gene sequence similarity has been used to identify a new species [50]. However, in many instances this was not sufficient for

species discrimination and a cutoff of 98.5% similarity has become the new 'gold-standard' [51, 52].

Other phylogenomic approaches for species discrimination (dDDH, ANI, AAI and Tetra)

Today, phylogenomic approaches such as dDDH, ANI (average nucleotide identity), AAI and Tetra (Tetra Nucleotide Signature Correlation Index) are frequently used for microbial classification and often provide better criteria for species discrimination than 16S rRNA gene sequence similarity [53]. The currently accepted cutoff values for delimiting species boundaries are about 70% for dDDH [41, 42], 95% for ANI [38, 54–56], 95–96% for AAI [57, 58] and 0.989 for Tetra [39, 59]. Using these approaches, we report the values for the comparisons between the three validated species of *Acidihalobacter* and strain F5[T] (Fig. 5). These values support the previously published species designations for *A. ferroxydans* DSM 14175[T], *A. aeolianus* DSM 14174[T] and *A. prosperus* DSM 5130[T] [14]. The values for the comparison of strain F5[T] with *A. prosperus* DSM 5130[T] are as follows (Fig. 5): 22.1 % (dDDH); 79.21 % (ANI); 83.93 % (AAI); and 0.92 (Tetra).
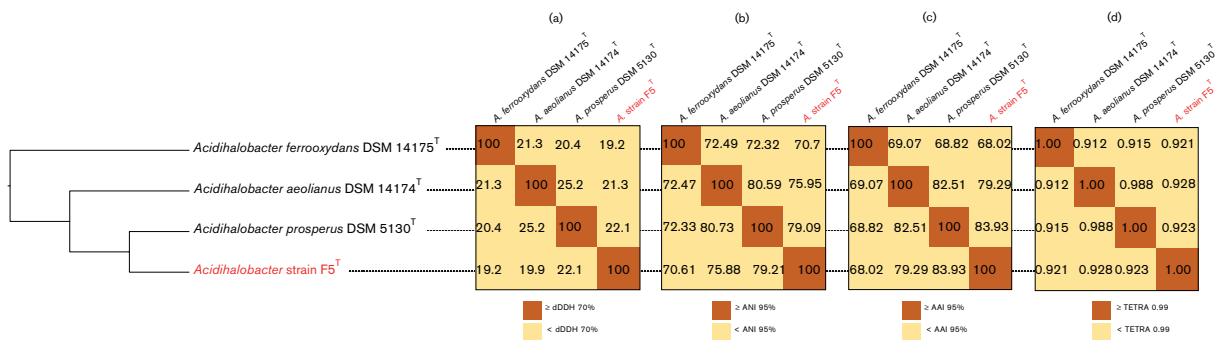
Fig. 5. Heat maps indicating the genetic relatedness between the three validated *Acidihalobacter* species and strain F5[T] displayed as a cladogram using different non-sequence-based methods. (a) dDDH, digital DNA–DNA hybridization, (b) ANI, average nucleotide identity, (c) AAI, average amino acid identity and (d) Tetra, nucleotide signature correlation index. The cutoffs for each of the four methods represent the values accepted as defining different species.

These results are all well below the accepted cutoff values for species delineation, indicating that strain F5^T should be considered as representing a new species of the genus *Acidihalobacter*.

DESCRIPTION OF *ACIDIHALOBACTER YILGARNENSIS* SP. NOV.

Acidihalobacter yilgarnensis (yil.garn.en′sis . N.L. masc. adj. *yilgarnensis*, referring to its isolation from the Yilgarn region, Western Australia).

Cells are Gram-stain-negative, motile, straight rods (1–2 µm long). Extremely acidophilic, optimum pH for growth is pH 2.5 with a range of pH 2.0–4.0. Halotolerant, can grow at up to 1283 mM NaCl with optimal growth at 428 mM NaCl. Mesophilic, optimal growth occurs at 30 °C, and capable of growth between 24 and 33 °C. Chemolithoautotrophic and aerobic. Able to utilize ferrous iron, elemental sulphur and tetrathionate as electron donors. It is able to leach base metals from the sulfide mineral pyrite (FeS$_2$) at up to 846 mM NaCl, pentlandite (Fe,Ni)$_9$S$_8$) at 1283 mM NaCl and chalcopyrite at 508 mM NaCl. Predicted terminal oxidases from the genome include aa_3 (EC 1.9.3.1), bo_3 (EC 1.10.3.10), *bd-I* (EC 1.10.3.14) and fumarate reductase (quinol, EC 1.3.5.1–1.3.5.4). Predicted respiratory quinones from the genome include ubiquinone (EC 1.14.13.-, 2.1.1.64, 2.1.1.63, 2.1.1.201, 2.1.1.222, 2.5.1.39, 2.5.1.129, 4.1.1.98). The genome contains a full compliment of *sox* genes distributed in two clusters (*soxXYZ* and *soxXA*) and separated *soxA* and *soxB*. It also includes a gene cluster for the predicted biosynthesis of the osmoprotectant ectoine. The G+C content of the DNA is 59.9 mol%. The genome contains one copy of both the 16S and 23S rRNA genes and contains 3233 coding sequences and 47 tRNA genes. The whole-genome sequence of 3566941 bp is available (GenBank accession no. CP017415.1).

The type strain is F5^T (=DSM 105917^T=JCM 32255^T), isolated from an acidic saline drain in the Yilgarn region, Western Australia.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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