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Acidibrevibacterium fodinaquatile gen. nov., sp. nov., isolated from acidic mine drainage

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Abstract

A heterotrophic and acidophilic bacterial strain, G45-3^T, was isolated from acidic mine drainage sampled in Fujian Province, PR China. Cells of strain G45-3^T were Gram-stain-negative, non-spore-forming, non-motile and rod-shaped. Catalase and oxidase activities were positive. Strain G45-3^T grew aerobically at 20–45 °C (optimum, 37 °C) and at pH 2.5–5.0 (optimum, pH 4.0). Photosynthetic pigments were not produced. Analysis of 16S rRNA gene sequences showed that strain G45-3^T was phylogenetically related to different members of the family *Acetobacteraceae*, and the sequence identities to *Acidisphaera rubrifaciens* JCM 10600^T, *Rhodovastum atsumiense* G2-11^T and *Rhodopila globiformis* ATCC 35887^T were 95.9, 95.3 and 95.3 %, respectively. Strain G45-3^T contained ubiquinone-10 as its respiratory quinone. The major polar lipids were determined to be diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminophospholipid and an unidentified aminolipid. The predominant fatty acids were cyclo-$C_{19:0}\omega8c$, $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{18:0}$. The genome of G45-3^T consists of one chromosome (3 907 406 bp) and three plasmids (68 344, 45 771 and 16 090 bp), with an average G+C content of 65.9 mol%. Based on the results of phenotypic and genomic analyses, it is concluded that strain G45-3^T represents a novel species of a new genus, for which the name *Acidibrevibacterium fodinaquatile* gen. nov., sp. nov. is proposed. *A. fodinaquatile* is nominated as type species and its type strain is G45-3^T (=CGMCC 1.16069^T=KCTC 62275^T).

The family *Acetobacteraceae* is a member of the class *Alphaproteobacteria* in the phylum *Proteobacteria* and was established in 1980 [1]. At the time of writing, 39 genera are validly named and are listed on the LPSN (www.bacterio.net/acetobacteraceae.html) [2]. According to their physiology and ecology, members of the family *Acetobacteraceae* can be basically classified into two groups, the acetate-producing bacteria and non-acetate-producing bacteria [3]. Acetate-producing *Acetobacteraceae* are described as obligate aerobes that oxidize sugars, sugar alcohols and ethanol to organic acids, aldehydes and ketones during incomplete oxidation processes [4] and comprise genera such as *Acetobacter*, *Gluconacetobacter*, *Gluconobacter*, *Granulibacter*, *Komagataeibacter*, *Kozakia*, *Acidimonas* and *Asaia*. These bacteria have two crucial enzymes, alcohol dehydrogenase and aldehyde dehydrogenase, and play important roles in beverage production and food fermentation [4–6]. The non-acetate-producing *Acetobacteraceae* mainly includes acidophilic or neutrophilic bacteria such as *Acidiphilium* [7], *Acidocella* [8], *Acidicaldus* [9, 10], *Acidisphaera* [11] and *Acidisoma* [12]. These bacteria phylogenetically form an independent lineage in the family *Acetobacteraceae* and are widely used in bioprocessing of minerals and bioremediation due to their versatile metabolisms [3]. In this study, we report the isolation from acidic mine drainage and the taxonomic position of strain G45-3^T, which was characterized as representing a novel mesophilic and obligately acidophilic genus, *Acidibrevibacterium*, within the family *Acetobacteraceae* based on its phenotypic, chemotaxonomic and genotypic features.

Strain G45-3^T was isolated from samples of acidic mine drainage from the Zijin copper mine area of Shanghang County, Fujian Province, PR China. The pH value of the acidic mine drainage samples was pH 1.7. For each enrichment step of acidophiles, 10 ml acidic mine drainage sample

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Keywords: *Acidibrevibacterium fodinaquatile*; Acetobacteraceae; acidic mine drainage; acidophile.
Abbreviations: ANI, average nucleotide identity; Bchl *a*, bacteriochlorophyll *a*; DDH, DNA–DNA hybridization; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.
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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene is KY777995. The genome of strain G45-3^T consists of one chromosome and three plasmids, their GenBank/EMBL/DDBJ accession numbers are CP029176, CP029177, CP029178 and CP029179, respectively.
Six supplementary figures and two supplementary tables are available with the online version of this article.

was inoculated into a 500 ml flask with 200 ml G4 medium and was incubated at 45 °C for 7–10 days. The G4 medium consisted of (per litre distilled water): 5 g glucose, 0.25 g yeast extract, 2 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.5 g $K_2HPO_4{\cdot}3H_2O$, 0.1 g KCl and 1 ml trace elements solution. The trace elements solution comprised (per litre distilled water): 1.8 mg $MnCl_2{\cdot}4H_2O$, 4.5 mg $Na_2B_4O_7{\cdot}10H_2O$, 0.22 mg $ZnSO_4{\cdot}7H_2O$, 0.05 mg $CuCl_2{\cdot}2H_2O$, 0.03 mg $Na_2MoO_4{\cdot}2H_2O$, 0.03 mg $VOSO_4{\cdot}2H_2O$ and 0.01 mg $CoSO_4$ [13]. For the preparation of solid plates, equal volumes of autoclaved, twice-concentrated G4 broth (pH adjusted to 4.0) and Gelrite (11 g l^{-1}; Sigma) solutions were mixed. After three enrichment steps, the cultures were serially diluted (tenfold) in tubes containing 0.9 ml G4 medium. Dilutions (0.2 ml) were then spread on the G4 solid plate. Strain G45-3^T was obtained from plate cultivation and further purified by repeated streaking on the G4 solid plates. *Acidisphaera rubrifaciens* JCM 10600^T was used as reference during this study [11]. Unless otherwise mentioned, strain G45-3^T and the reference strain, JCM 10600^T, were cultured in either G4 or J270 medium (prepared according to www.jcm.riken.jp/cgi-bin/jcm). Cell growth was estimated by measuring turbidity at 600 nm (OD_{600}) using a UV/visible spectrophotometer (SPECORD205, Analytik Jena).

Genomic DNA was extracted and purified according to the method described previously [14]. The 16S rRNA genes were amplified by PCR using primers 27F and 1492R [15] and sequenced. The genome of strain G45-3^T was sequenced using the PacBio RS II and Illumina HiSeq 4000 platforms at the Beijing Genomics Institute (BGI; Shenzhen, PR China). Four SMRT cell zero-mode waveguide arrays of sequencing were used by the PacBio platform to generate the subread set. Subreads of length <1 kb were removed. Genome contamination was checked with ContEst16S (www.ezbiocloud.net/tools/contest16s). Self-correction proceeded with Pbdagcon (https://github.com/PacificBiosciences/pbdagcon). The complete genome was assembled using the Celera assembler against a high-quality corrected circular consensus sequence subread set. To improve the accuracy of the genome sequences, GATK (www.broadinstitute.org/gatk/) and SOAP tool packages (SOAP2, SOAPsnp, SOAPindel) were used to make single-base corrections [16, 17]. To trace the presence of any plasmid, the filtered Illumina reads were mapped using SOAP to the bacterial plasmid database (www.ebi.ac.uk/genomes/plasmid.html).

Cell morphology and flagella were visualized after cultivation on G4 and J270 solid plates for 7–14 days at 37 °C with transmission electron microscopy (JEM-1400, JEOL). The temperature range for growth was determined at different temperatures (4, 15, 25, 30, 37, 40 and 45 °C). The growth at various pH values of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 10.0, 11.0 and 12.0 was tested by adjusting the pH with 1.0 mmol l^{-1} H_2SO_4 for pH 0.5–2.5) citrate/NaH_2PO_4 (for pH 3.0–5.5), phosphate (for pH 6.0–7.5) and Tris buffer (for pH 8.0–10) [18], or with 5 M NaOH (for pH 10.0–12.0). NaCl tolerance was measured in G4 broth supplemented with 0–6 % (w/v) NaCl at intervals of 1 %. Cellular growth was determined by measuring the OD_{600} of cultures grown at various temperatures, pH values and NaCl concentrations.

Cells of strain G45-3^T were Gram-stain-negative, non-motile, non-spore-forming, rod-shaped, 3.2–3.3 µm long and 1.3–1.5 µm wide (Fig. S1, available in the online version of this article). A range of homologous genes to flagellar assembly, such as *flgD*, *flgE*, *flgG*, *flgH*, *flgI* and *filC*, were identified from its complete genome. Colonies of strains G45-3^T on G4 and J270 plates were light yellow, flat and circular after 7 days of incubation at 37 °C. Strain G45-3^T grew within a pH range of pH 2.5–5.0 (optimum, pH 4.0) and within a temperature range of 20–45 °C (optimum, 37 °C). Strain G45-3^T grew at 0–1 % (w/v) NaCl. No growth occurred at NaCl concentrations higher than 1 %.

Utilization of carbon sources was carried out on G4 basal salt medium without adding glucose, to which the filter-sterilized stock solutions of carbohydrates were supplemented to the final concentration of 2.0 g l^{-1}. Aerobic growth was determined with G4 or J270 media with a shaker at 37 °C under darkness or with an illumination incubator at 2000 lux. Anaerobic growth under darkness or in the light was observed with Hungate tubes containing G4 or J270 broth supplemented with 0.5 g l^{-1} L-cysteine monohydrochloride and 1 ml 0.1 % (v/v) resazurin stock solution. Aerobic and anaerobic growth were also tested on G4 basal salt medium (without glucose) supplemented with sodium sulfide (10.0 g l^{-1}), elemental sulfur (5.0 g l^{-1}), sodium thiosulfate (10.0 g l^{-1}), potassium tetrathionate (3.0 g l^{-1}) or $FeSO_4$ (7.5 g l^{-1}). Oxidase and catalase activities were determined as described by Dong and Cai [19]. Hydrolyses of starch, casein and Tween 80 were tested as described by Lányí [20]. Nitrate reduction, indole production, hydrolyses of gelatin and aesculin, and assimilation of different substrates were tested with API 20NE strips (bioMérieux). The activities of various enzymes and acid formation from sugar were characterized by using API ZYM and API 50CH test strips (bioMérieux). All the experiments using commercial kits were performed according to manufacturer's instructions.

Strain G45-3^T grew exclusively under aerobic conditions; no growth was observed under anaerobic conditions with or without light. Oxidase and catalase activities were observed. Nitrate was reduced to nitrite. Indole production was negative. Hydrolyses of L-arginine and urea were positive, but hydrolyses of starch, Tween 80, casein or aesculin was negative. Alkaline phosphatase, esterase (C4) and esterase lipase (C8) production was positive. Growth was observed with L-arabinose, ethanol, D-galactose, D-glucose, maltose, D-mannose, D-ribose and D-xylose. Genome data-mining revealed a range of possible metabolic pathways responsible for the utilization of various sugars in strain G45-3^T such as glycolysis, pyruvate metabolism and fermentation. D-Fructose, inositol, lactose, D-mannitol, D-sorbitol and sucrose were not assimilated.

Table 1. Phenotypic characteristics that differentiate the novel genus *Acidibrevibacterium* from its closely related genera *Acidisphaera*, *Rhodopila* and *Acidosoma*. A detailed description of results from the API ZYM, API 20NE and API 50CH test kits is provided as Table S1

Genera: 1, *Acidibrevibacterium*; 2, *Acidisphaera* (data are from the present study, except the G+C content of JCM 10600^T is taken from Hiraishi *et al.* [11]); 3, *Rhodopila* [11]; 4, *Rhodovastum* [21]; 5, *Acidicaldus* [9, 10]; 6, *Acidisoma* [12]; 7, *Acidocella* [8]; 8, *Acidiphilium* [7]; 9, *Granulibacter* [45]; 10, *Endobacter* [44]. +, Positive; −, negative; w, weakly positive. For carbon source utilization: +, moderate growth (OD_{660}=0.1–0.5); [+], poor growth (OD_{660}=0.05–0.1); +/−, little or ambiguous growth; −, no growth; ND, no data.

Characteristic	1	2	3	4	5	6	7	8	9	10
Cell shape	Rods	Cocci, short rods	Cocci	Ovals, rods	Rods	Coccobacilli	Rods, coccobacilli	Rods	Coccobacilli, rods	Cocci, rods
Cell size (μm)	1.3–1.5×3.2–3.3	0.7–0.9×0.9–1.6	1.6–1.8	2.0–3.0×3.0–10	0.5–0.8×1.2–4.0	0.7–1.5×1.4–4.1	0.5–0.8×1.0–2.0	0.3–1.2×4.2	ND	ND
Colony colour	Light yellow	Salmon pink	Purple-red	Red-brown	Off white/cream	White, cream	White, cream, light brown	Pink, red	Yellow	White, mucoid
Motility	−	−	+	+	+	−	+	+	−	+
Anaerobic phototrophy	−	−	+	+	−	−	−	−	−	−
Bacteriochlorophyll *a*	−	+	+	+	−	−	−	+	ND	−
Carotenoid	−	+	+	+	−	−	−	−	ND	−
Temperature range for growth (°C)	20–45	20–40	ND	20–40	45–60	2–30	25–37	20–47	25–37	20–37
Optimal growth temperature (°C)	37	30–35	30–35	30–35	53–55	18–22	ND	27–30	35–37	28
pH range for growth	2.5–5	3.5–6	4.2–6.5	5.0–8.5	1.8–3.0	3.7–7.5	3.0–6.0	1.9–5.9	3.5–6.5	3.5–7.0
pH optimum for growth	4.0	4.5–5.0	4.8–5.0	6.0–6.5	2.5–3.0	5.0–5.7	ND	3.2–3.5	5.0–6.5	5.0–7.0
Tolerance of>2.5 (%, w/v) NaCl	−	+	+	−	ND	−	+	+	ND	ND
Growth on methanol	+	−	−	−	+	−	−	−	+	−
Major quinone(s)	Q-10	Q-10	Q-9(10), MK-9 (10), RQ-9	Q-10, RQ-10	ND	Q-10	Q-10	Q-10	ND	Q-10
Major fatty acids	Cyclo-$C_{19:0}\omega8c$, $C_{18:1}$, $C_{16:0}$	Cyclo-$C_{19:0}\omega8c$, $C_{18:1}$, $C_{16:0}$	$C_{18:1}$	$C_{18:1}$	ND	Cyclo-$C_{19:0}\omega8c$	$C_{18:1}$	$C_{18:1}$	$C_{18:1}$, $C_{16:0}$	Cyclo-$C_{19:0}\omega8c$, $C_{18:1}$, $C_{16:0}$
Unidentified aminophospholipid	+	−	ND	ND	ND	−	ND	ND	ND	+
DNA G+C content (mol %)	65.9	69.1–69.8	66.3	67.8	70.9–72.7	60.5–61.9	58.7–64.4	62.9–68.3	59.1	60.3

Other physiological and biochemical characteristics of strain G45-3^T are presented in the species description and in Table S1. The differences from its close phylogenetically related species of the genera *Acidisphaera* [11], *Rhodovastum* [21], *Rhodopila* [22] and *Acidosoma* [12] are detailed in Table 1. As indicated in Table 1, strain G45-3^T was obviously different from its close neighbours, *Acidisphaera*, *Rhodopila* and *Acidisoma*, by cell shape, colony pigmentation, pH and temperature optimum (Table 1).

BChl *a* and carotenoids were extracted from cells of both strain G45-3^T and *A. rubrifaciens* JCM 10600^T with acetone/methanol (7 : 2,v/v) and were detected with UV/visible spectrophotometer (SPECORD205, Analytik, Jena) [23]. Extracts from *A. rubrifaciens* JCM 10600^T were used as references. Spectroscopic experiments showed that the acetone:methanol extracts of the strain G45-3^T had no absorption peaks at 412, 496–504, 540–550 and 873–874 nm (Fig. S2), suggesting that strain G45-3^T did not produce BChl *a* and carotenoids either in darkness or under incandescent illumination. Genome data-mining revealed that strain G45-3^T missed key genes for BChl *a* and carotenoid biosynthesis (Fig. S3). Strain G45-3^T was distinguishable from *Rhodopila*, *Rhodovastum* and *Acidisphaera* by production of BChl *a* and carotenoid production.

For the profiling of cellular fatty acids, respiratory quinones and polar lipids, cells of G45-3^T and of *A. rubrifaciens* JCM 10600^T grown on G4 and J270 plates were harvested after 7–14 days growth. The cells were washed twice and resuspended in 50 mM phosphate buffer (pH 7.0), sonicated for 3 min and centrifuged to remove unbroken cells. Cellular fatty acids were extracted, saponified and methylated according to the standard protocol of Sherlock Microbial

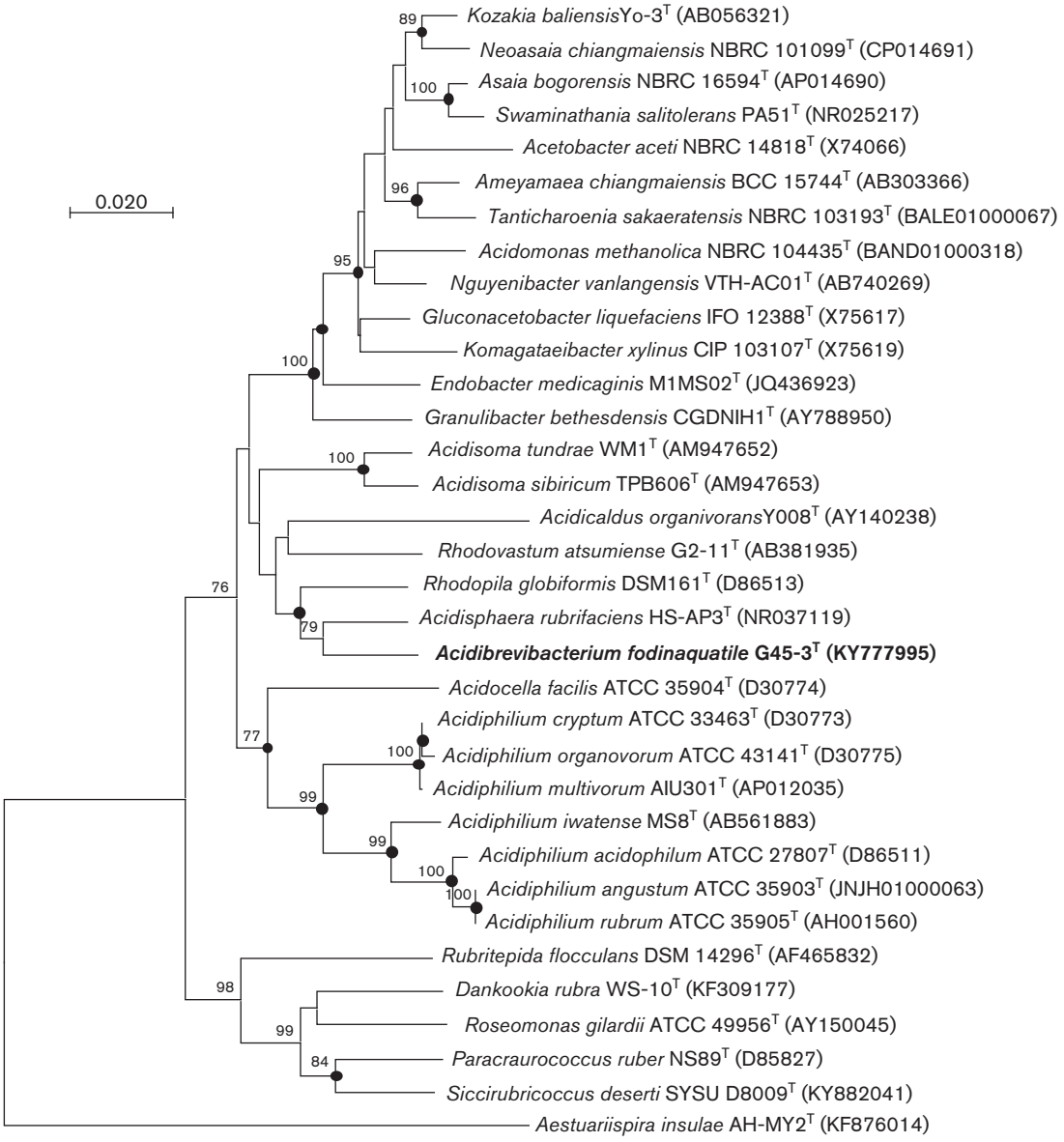

Fig. 1. Phylogenetic tree reconstructed with the neighbour-joining method based on 16S rRNA gene sequences of strain G45-3^T and type species of the family *Acetobacteraceae*. The species within the sublineage clustered closely with G45-3^T are compared in more details of their phenotypes. *Aestuariispira insulae* AH-MY2^T (KF876014) was used as an outgroup. Numbers at branch nodes represent confidence levels (values>70 % are shown) from 1 000 replicate bootstrap samplings. Filled circles indicate branches that are also found in trees generated with the maximum-parsimony and the maximum-likelihood algorithms. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position.

Identification System version 6.0 (MIDI), analysed by gas chromatography (HP 6890 Series GC System, Agilent) and identified using the TSBA6 database of the Microbial Identification System [24]. The respiratory quinones were extracted with chloroform/methanol (2 : 1, v/v) from freeze-dried cells (600 mg) and were fractionated by TLC. The purified quinones were identified by HPLC equipped with a ZOBAX ODS C18 column (4.6×250 mm; Agilent) as described previously [25]. Polar lipids were extracted as described by Minnikin *et al.* [26] and separated by two-dimensional TLC (Merck silica gel 60; 10×10 cm), and identified by spraying with appropriate detection reagents. Total lipids profiles were stained with 10 % (w/v) ethanolic molybdatophosphoric acid (Sigma-Aldrich), aminolipids were detected with 0.4 % (w/v) solution of ninhydrin (Sigma Life Science) in butanol, phospholipids were detected with Zinzadze reagent (molybdenum blue spray reagent, 1.3 %;

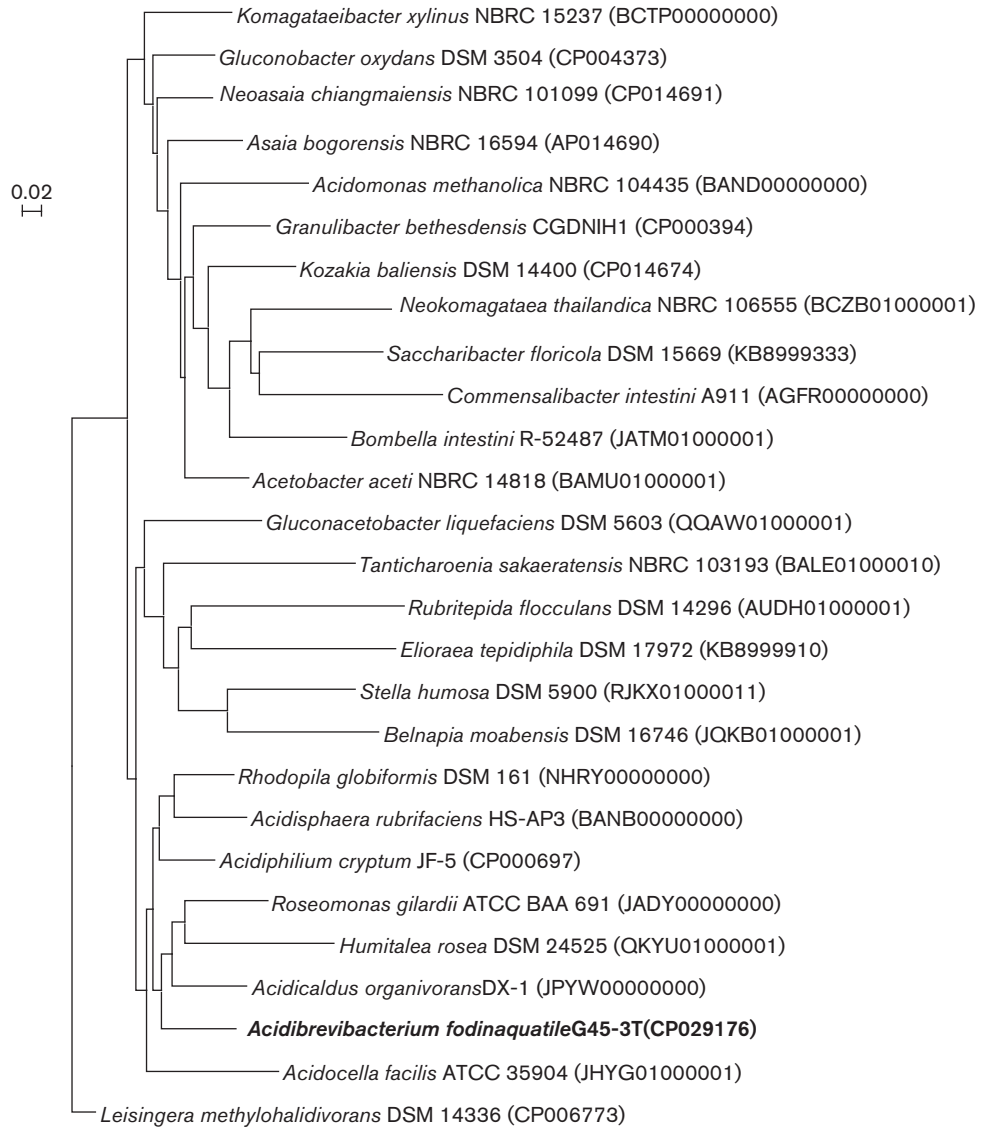

Fig. 2. Phylogenetic tree reconstructed using the composition vector approach (CVTree 3.0) based on whole-genome sequences of strain G45-3^T and the type species within the family *Acetobacteraceae*. *Leisingera methylohalidivorans* DSM 14336 (CP006773) was used as an outgroup. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position. In this CVTree, the branching reflects taxonomy and the length represents evolution.

Sigma Life Science) and glycolipids were detected with α-naphthol reagent (0.5 %, w/v).

Predominant cellular fatty acids (>5 %) of strain G45-3^T were cyclo-$C_{19:0}\omega 8c$ (31.7 %), $C_{18:1}\omega 7c$ (24.1 %), $C_{16:0}$ (22.0 %) and $C_{18:0}$ (9.4 %). The detailed fatty acid compositions of G45-3^T and its closest relatives are listed. The predominant quinone of strain G45-3^T was ubiquinone-10, which was the same as the *Acidisphaera* and *Acidosoma*, but was different from *Rhodopila* [22]. Strain G45-3^T displayed a polar lipid profile consisting of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminophospholipid, and an unidentified aminolipid (Fig. S4). Strain G45-3^T was different from *A. rubrifaciens* JCM 10600^T in that an aminophospholipid presented in strain G45-3^T, but did not in strain JCM 10600^T.

The almost-complete 16S rRNA gene sequence of strain G45-3^T was obtained and the close phylogenetic neighbours were identified through a calculation performed using EzBioCloud [27]. The 16S rRNA gene sequences of strain G45-3^T (1 418 bp; GenBank accession no. KY777995) and the type strains of validly published type species of the family *Acetobacteraceae* were aligned using CLUSTAL X 2.1 [28]. Evolutionary divergence between type strains of validly

published type species of the family *Acetobacteraceae* was estimated by MEGA version 7.0 [29]. Phylogenetic analysis was carried out using the neighbour-joining (NJ) [30], maximum-parsimony (MP) [31] and maximum-likelihood (ML) [32] methods. The NJ tree was generated using Kimura's two-parameter method [33]. The MP tree was reconstructed using tree-bisection–reconnection. According to the MODELTEST scores, the Tamura–Nei model along with gamma distribution and invariant sites (TN93+G+I) fit the best, therefore, these parameters were used for the ML analysis. Branching patterns of trees were evaluated by bootstrapping with 1000 replications [34].

The 16S rRNA gene sequence analysis indicated that strain G45-3^T was most closely related to *Acidisphaera rubrifaciens* JCM 10600^T (95.9 %), *Rhodovastum atsumiense* G2-11^T (95.3 %), *Rhodopila globiformis* ATCC 35887^T (95.3 %) and *Acidisoma tundrae* WM1^T (94.6 %). Furthermore, phylogenetic trees reconstructed using the NJ algorithm displayed that strain G45-3^T formed a sublineage outside of the genera *Acidisphaera*, *Rhodovastum*, *Rhodopila*, *Acidisoma* and *Acidicaldus* (Fig. 1). The topology of the NJ tree was supported by those of the MP (Fig. S5) and ML trees (Fig. S6).

To analyse the evolution divergence based on whole-genome sequences, a phylogenomic tree was reconstructed using the composition vector approach (CVTree 3.0) [35, 36]. Genome sequences of strain G45-3^T and the type species of 25 genera within the family *Acetobacteraceae* from the NCBI (www.ncbi.nlm.nih.gov/) were used. The result indicated that strain G45-3^T also formed a sublineage outside of the genera *Rhodopila*, *Acidisphaera*, *Acidiphilium*, *Roseomonas*, *Humitalea* and *Acidicaldus*, although the topology was slightly different from the phylogenetic tree based on the 16S rRNA gene sequences (Fig. 2).

Moreover, average nucleotide identity (ANI) was analysed with whole-genome sequences by using the OrthoANI system [37]. Pairwise ANI values between strain G45-3^T and *Acidisphaera rubrifaciens* JCM 10600^T and *Rhodopila globiformis* ATCC 35887^T were 73.3 and 72.1 %, much less than the generally accepted species boundary of 95 % [38, 39]. The digital DNA–DNA hybridization (DDH) and G+C difference values between strain G45-3^T and two closely related genus were calculated using the GGDC (Genome-to-Genome Distance Calculator, https://ggdc.dsmz.de/), and results showed that strain G45-3^T had DDH and G+C differences of 14 % and 4.71 mol%, respectively, from *Acidisphaera rubrifaciens* JCM 10600^T and of 13.4 % and 1.52 mol% from *Rhodopila globiformis* ATCC 35887^T, which showed the genomic differences among members of different genera of the family *Acetobacteraceae*.

The N50 of the filtered reads was 12 007 bp. The complete genome of G45-3^T was 4 037 611 bp long and composed of one chromosome (3 907 406 bp) and three plasmids (68 344, 45 771 and 16 090 bp, respectively). There is only one copy of the 16S rRNA gene in the chromosome. One full-length 16S rRNA gene copy (1 485 bp) was extracted from the whole G45-3^T genome, and it showed 99.9 % identity to the PCR-amplified 16S rRNA gene (1 418 bp), which ensured the authenticity of genome data. The assembled genome has a depth of 142× and consisted of 3966 coding sequences, of which 90.4 % were assigned to known functional genes using Glimmer (G.3.03) [40–42]. There were 68 ncRNA genes in the G45-3^T genome. One possible CRISPR was predicted in G45-3^T chromosome. The DNA G+C contents were determined by both the thermal denaturation method [43] and calculation of genome data. Results showed that the DNA G+C content of strain G45-3^T was 63.8 mol%, as determined with the thermal denaturation method. The G+C content of strain G45-3^T was calculated to be 65.9 mol% according to its genome data.

In summary, strain G45-3^{T}isolated from acidic mine water possessed a number of characteristics that clearly distinguished it from phylogenetically related members of the family *Acetobacteraceae*. It could be differentiated from its most closely related phylogenetic neighbour *Acidisphaera rubrifaciens* based on a number of phenotypic traits, such as the absence of Bchl *a* and carotenoid, growth on methanol, NaCL sensitivity, and the presence of unidentified aminophospholipid in its polar lipid profile. The absence of photopigments and a more complicated fatty acid profile distinguished it from members of the genera *Rhodopila*, *Rhodovastum*, and *Acidiphilium*. It could be differentiated from members of the genera *Acidicaldus*, *Acidisoma* and *Endobacter* based on different optimum growth temperature and pH [44]. Fatty acid composition and sensitivity of NaCl distinguished it from the genera *Acidocella* and *Granulibacter* [45]. On the basis of the polyphasic data, including morphology, biochemical and phylogenetic characteristics, DNA G+C content, genome data, cellular fatty acids, and polar lipid profiles, strain G45-3^T is considered to represent a novel species of a new genus *Acidibrevibacterium*, for which the name *Acidibrevibacterium fodiniaquatile* gen. nov., sp. nov. is proposed.

DESCRIPTION OF *ACIDIBREVIBACTERIUM* GEN. NOV.

Acidibrevibacterium (a.ci.di.bre.vi.bac.te′ri.um. N.L. neut. n. *acidium* an acid; L. masc. adj. *brevis* short; N.L. neut. n. *bacterium*, rod; N.L. neut. n. *Acidibrevibacterium* acid-requiring short rod micro-organism).

Cells are non-motile straight rods. Non-spore-forming. Gram-stain-negative. Obligately aerobic chemo-organotroph. BChl *a* and carotenoids are not produced either in darkness or under incandescent illumination. Mesophilic and obligately acidophilic. Ubiquinone-10 is the main respiratory quinone. The polar lipid profile includes diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unidentified aminophospholipid and aminolipid. The major fatty acids are cyclo-$C_{19:0}\omega 8c$, $C_{18:1}\omega 7c$, $C_{16:0}$ and $C_{18:0}$. The G+C content of the genomic DNA ranges from 63.8 to

65.9 %. Member of the family *Acetobacteraceae*. The type species is *Acidibrevibacterium fodinaquatile*.

DESCRIPTION OF *ACIDIBREVIBACTERIUM FODINAQUATILE* SP. NOV.

Acidibrevibacterium fodinaquatile (fo.din.a.qua′ti.le. L. fem. n. *fodina* mine; L. masc. adj. *aquatilis* living in water; N.L. neut. adj. *fodinaquatile* living in mine water).

The description is as for the genus and with the following additional traits. Cells are non-motile rods measuring 1.3–1.5 µm wide and 3.2–3.3 µm long. Growth occurs at temperature range of 20–45 °C (optimum, 37 °C) and a pH range of pH 2.5–5.0 (optimum, pH 4.0), and tolerates less than 1 % (w/v) of NaCl. Catalase- and oxidase-positive. DL-Arabinose, D-ribose, DL-xylose, D-galactose, D-glucose, L-rhamnose, D-lyxose, DL-fucose and potassium gluconate can be utilized for acid production. Nitrate is reduced to nitrite. L-Arginine and urea are hydrolysed, but starch, Tween 80, gelatin, casein and aesculin are not. Glucose fermentation is positive. D-Glucose and L-arabinose are assimilated and maltose is weakly assimilated. D-Mannose, D-mannitol, *N*-acetyl-glucosamine, malate, trispdium citrate and phenylacetic acid cannot be assimilated. In API ZYM strip tests, production of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysine arylamidase, α-chymotrypsin, acid phosphatase and phosphohydrolase is positive. For the utilization of carbon sources, moderate growth is observed with galactose, D-glucose, D-xylose and ethanol, poor growth is observed with L-arabinose, methanol, propanol, alanine, asparagine and aspartate, no growth occurs on glycerol, D-mannitol, D-mannose, butanol, malate, pyruvate, fumarate, succinate and casamino acids.

The type strain is G45-3^{T} (=CGMCC 1.16069^{T}=KCTC 62275^{T}), isolated from acidic mine drainage samples. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene is KY777995. The genome of the type strain is 4 037 611 bp and consists of one chromosome (3 907 406 bp) and three plasmids (68 344, 45 771 and 16 090 bp, respectively). The GenBank/EMBL/DDBJ accession numbers are CP029176, CP029177, CP029178 and CP029179, respectively. The DNA G+C content is 65.9 mol%, as calculated from genome data.

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

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