Arenimonas metalli sp. nov., isolated from an iron mine

Fang Chen, Zunji Shi and Gejiao Wang

State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan, 430070, PR China

Correspondence
Gejiao Wang
gejiao@mail.hzau.edu.cn

A Gram-staining-negative, aerobic, rod-shaped bacterium (CF5-1^T) was isolated from Hongshan Iron Mine, Daye City, Hubei province, China. The major cellular fatty acids (>10 %) were iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and iso-$C_{17:1}\omega9c$. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major respiratory quinone was Q-8. The genomic DNA G+C content was 70.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CF5-1^T was most closely related to *Arenimonas malthae* (95.3 % gene sequence similarity), *Arenimonas oryziterrae* (94.7 %), *Arenimonas donghaensis* (94.6 %) and *Arenimonas composti* (94.5 %). A taxonomic study using a polyphasic approach showed that strain CF5-1^T represents a novel species of the genus *Arenimonas*, for which the name *Arenimonas metalli* sp. nov. is proposed. The type strain is CF5-1^T (=CGMCC 1.10787^T=KCTC 23460^T=CCTCC AB 2010449^T).

The family *Xanthomonadaceae* was described by Saddler & Bradbury (2005), and although, according to Rule 51b(1) of the *Bacteriological Code* (1990 Revision; Lapage *et al.*, 1992), the name of this family is illegitimate because it contains the genus *Lysobacter*, many new strains have still been classified within this family (Johansen *et al.*, 2005; Jin *et al.*, 2007; Aslam *et al.*, 2009; Ten *et al.*, 2009; Lee *et al.*, 2011). To date, there are 20 genera in the family *Xanthomonadaceae*, including several genera that were established in recent years, *Arenimonas* (Kwon *et al.*, 2007), *Ignatzschineria* (Tóth *et al.*, 2007), *Wohlfahrtiimonas* (Tóth *et al.*, 2008), *Rudaea* (Weon *et al.*, 2009) and *Pseudofulvimonas* (Kämpfer *et al.*, 2010). The genus *Arenimonas* was first proposed by Kwon *et al.* (2007). At the time of writing, the genus *Arenimonas* contained only four species with validly published names, *Arenimonas donghaensis* (type species) (Kwon *et al.*, 2007), *Arenimonas malthae* (Young *et al.*, 2007), *Arenimonas oryziterrae* (Aslam *et al.*, 2009) and *Arenimonas composti* (Aslam *et al.*, 2009), isolated from seashore sand, oil-contaminated soil, the rice rhizosphere and compost, respectively. The species *Arenimonas composti* (Aslam *et al.*, 2009) was previously classified as *Aspromonas composti* (Jin *et al.*, 2007). The common characteristics of members of the genus *Arenimonas* are Gram-staining-negative, aerobic, rod-shaped, non-spore-forming, oxidase-positive, non-indole-producing, non-nitrate-reducing, containing iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$ as the major fatty acids, phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) as the major polar lipids, Q-8 as the major respiratory quinone, and possessing relatively high DNA G+C content (65.0–70.8 mol%) (Kwon *et al.*, 2007; Young *et al.*, 2007; Jin *et al.*, 2007; Aslam *et al.*, 2009). Of these characteristics, Gram-staining-negative, aerobic, rod-shaped, non-spore-forming and Q-8 as the major respiratory quinone are also common among the genera within the family *Xanthomonadaceae* (Busse *et al.*, 2002; Lee *et al.*, 2005; Yoon *et al.*, 2006; Tóth *et al.*, 2008).

In order to investigate culturable bacteria from an iron mine environment, iron mining powder was collected from Hongshan Iron Mine (about 100 m underground, 30° 04′ 38.77″ N, 114° 57′ 24.07″ E), Daye City, Hubei Province, China. The pH (in water) of the iron mining powder was 7.85. The total C, N, P, S and Fe concentrations were 11.61, 0.21, 0.13, 6.37, 308.84 g kg^{-1}, respectively, as determined by atomic absorption spectroscopy (AAS). Bacterial isolation was performed using R2A (Difco) agar plates incubated at 28 °C for 7 days. In this study, we report the taxonomic classification of strain CF5-1^T.

For analyses of morphological, physiological and biochemical characteristics, strain CF5-1^T and four members of the genus *Arenimonas*, *A. malthae* CCUG 53596^T, *A. oryziterrae* KCTC 22247^T, *A. donghaensis* DSM 18148^T and *A. composti* KCTC 12666^T, were cultivated on R2A agar or broth and

Abbreviations: DPG, Diphosphatidylglycerol; MIC, minimum inhibitory concentration; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipids; PME, phosphatidylmonomethylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CF5-1^T is HQ698842.

Three supplementary figures are available with the online version of this paper.

incubated at 28 °C for 3 days unless otherwise stated. Cell morphology and the presence of flagella were observed using a transmission electron microscope with cells grown for 1 day at 28 °C in half-strength R2A broth (Aslam *et al.*, 2009). Colony morphology was observed on R2A agar. Gram-staining was performed as described by Dussault (1955) combined with the KOH lysis method (Ryu, 1938). Growth at different temperatures (0, 4, 15, 20, 28, 37 and 42 °C) was tested on R2A agar for 2 weeks. Salt tolerance was tested in R2A broth supplemented with 0–5 % (w/v) NaCl incubated at 28 °C for 7 days. Growth at pH 4–10 (using increments of 1 pH unit) was assessed after incubation in R2A broth at 28 °C for 7 days. The R2A broth was adjusted to initial pH value with citrate/phosphate buffer or Tris/hydrochloride buffer (Breznak & Costilow, 1994). Anaerobic growth was determined by incubation in an anaerobic chamber (Mitsubishi Gas Chemical Co, Inc.) for 2 weeks. Motility tests used R2A broth with 0.3 % agar and stab inoculation. Growth on nutrient agar, 1.0 % (w/v) trypticase soy broth (TSB) plus 1.5 % agar, MacConkey agar, full-strength trypticase soy agar (TSA) and Luria–Bertani (LB) agar (all from Difco) were investigated. Nitrate reduction was tested by the method described by Lányí (1987). Tests to determine indole production, catalase and oxidase activities, and hydrolysis of DNA were performed as described by Smibert & Krieg (1994). Hydrolysis of casein, aesculin, gelatin, L-tyrosine, urea, starch, Tween 80 and CM-cellulose were performed as described by Cowan & Steel (1965). Acid production from various carbohydrates was determined according to Hugh & Leifson (1953). Antibiotic-susceptibility tests were performed by spreading bacterial suspensions on culture plates and applying filter-paper discs containing different antibiotics. Antibiotic susceptibility was confirmed when an inhibition zone diameter was above 10 mm (Hangzhou Microbial Reagent Co., Ltd). Other physiological/biochemical properties and enzyme activities were examined using API 20NE and API ZYM systems (bioMérieux) according to the manufacturer's instructions. The single carbon substrate utilizations were determined using the GN2 MicroPlate (Biolog) according to the manufacturer's instructions. All strains were cultivated on R2A agar and incubated at 28 °C for 3 days.

The minimum inhibitory concentration (MIC), defined as the lowest metal(loid) concentration that completely inhibited the growth of strain CF5-1^T, was determined as described by Lim & Cooksey (1993). Triplicate samples of each single bacterial colony were grown overnight at 28 °C with 160 r.p.m. shaking. Then 2 % original culture was inoculated into 5 ml R2A broth with serial concentrations of $C_8H_4K_2O_{12}Sb_2 . 3H_2O$, $NaAsO_2$, $ZnSO_4 . 7H_2O$, $CuSO_4 . 5H_2O$, $NiCl_2 . 6H_2O$, $CoCl_2 . 6H_2O$, K_2CrO_4 and $FeCl_3 . 6H_2O$, respectively. The growth of strain CF5-1^T was measured at OD_{600} after incubation at 28 °C, 160 r.p.m. for 7 days.

A nearly complete 16S rRNA gene sequence was amplified as described by Fan *et al.* (2008) and compared with the sequences available in NCBI GenBank database using the BLASTN program. The similarities were calculated using the NCBI alignment and EzTaxon program (Chun *et al.*, 2007). The 16S rRNA gene sequence of strain CF5-1^T was aligned with the four type strains of members of the genus *Arenimonas*, some representatives of the family *Xanthomonadaceae* and *Escherichia coli* ATCC 11775^T (used as an outgroup) using the CLUSTAL_X program (Thompson *et al.*, 1997). A neighbour-joining (Saitou & Nei, 1987) tree and a maximum-parsimony (Fitch, 1971) tree were constructed using MEGA 4.0 (Tamura *et al.*, 2007). A maximum-likelihood tree was generated using the PHYML online web server (Guindon *et al.*, 2005). Bootstrap values were calculated based on 1000 replications (Felsenstein, 1985) in order to obtain the confidence level of the branches.

The DNA G+C content was determined by HPLC as described by Mesbah *et al.* (1989). For whole-cell fatty acid analysis, strain CF5-1^T and the four reference strains were grown in R2A broth at 28 °C until they reached exponential phase and were then analysed by GC (6890; Hewlett Packard), according to the instructions of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10) (Kroppenstedt, 1985; Sasser, 1990). The respiratory quinones were extracted and identified by HPLC as described by Xie & Yokota (2003). Polar lipids were determined by two-dimensional TLC as described by Tindall (1990) and Ventosa *et al.* (1993).

Strain CF5-1^T was Gram-staining-negative, aerobic, non-motile and non-spore-forming. A transmission electron micrograph showed that the cell morphology was rod-shaped without flagella (Fig. S1, available in IJSEM online). Colonies on R2A agar plates were smooth, circular, yellowish to creamy white and 1–2 mm in diameter. The strain grew well on R2A agar, nutrient agar, 1.0 % (w/v) TSB plus 1.5 % agar, full-strength TSA and LB agar, but did not grow on MacConkey agar. Detailed results of the polyphasic characteristics of strain CF5-1^T are given in the species description. The main differences in phenotypic characteristics between strain CF5-1^T and the four reference species of the genus *Arenimonas* are shown in Table 1. In addition, the MICs of strain CF5-1^T were 0.1, 0.1, 0.1, 0.3, 0.3, 0.4, 0.5 and 0.5 mM for Sb^{3+}, As^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Cr^{6+} and Fe^{3+}, respectively.

The 1506 bp 16S rRNA gene sequence of strain CF5-1^T was closely related to *A. malthae* CC-JY-1^T, *A. oryziterrae* YC6267^T, *A. donghaensis* HO3-R19^T and *A. composti* TR7-09^T, with nucleotide similarities of 95.3 %, 94.7 %, 94.6 % and 94.5 %, respectively. Lower gene sequence similarities ($\leqslant$94.1 %) were found with the other genera of the family *Xanthomonadaceae*. A phylogenetic tree constructed using the neighbour-joining algorithm grouped strain CF5-1^T in the same cluster with the four species of the genus *Arenimonas* and separated from other genera of the family *Xanthomonadaceae* (Fig. 1). Analyses using the maximum-parsimony and the maximum-likelihood algorithms showed similar results (Fig. S2a, b).

Table 1. Differential phenotypic characteristics between strain CF5-1^T and related members of the genus *Arenimonas*

Strains: 1, CF5-1^T; 2, *A. malthae* CCUG 53596^T; 3, *A. oryziterrae* KCTC 22247^T; 4, *A. donghaensis* DSM 18148^T; 5, *A. composti* KCTC 12666^T. All data are from this study except for isolation sources. +, Positive; −, negative; w, weak reaction.

Characteristic	1	2	3	4	5
Isolation source	Iron mine	Oil-contaminated soil	Rice rhizosphere	Seashore sand	Compost
Motility	−	+	−	+	+
Temperature range (°C)	4–37	15–36	15–37	4–37	20–42
NaCl range (%, w/v)	0–1	0–2	0	0–3	0–2
Catalase	+	+	+	+	−
Hydrolysis of:					
Cellulose	−	−	+	−	−
DNA	+	+	−	+	+
Arginine	−	+	−	−	−
Enzyme activities					
Lipase (C14)	+	−	+	−	−
Valine arylamidase	−	−	+	−	−
Cystine arylamidase	−	−	+	−	−
Trypsinase	+	+	+	+	−
Assimilation of:					
Dextrin	−	−	−	−	+
Sucrose	−	w	−	−	−
Pyruvic acid methyl ester	+	w	+	−	−
β-Hydroxybutyric acid	+	+	−	+	+
L-Alaninamide	+	w	+	w	−
L-Alanine	−	w	+	−	−
L-Alanyl glycine	+	w	+	−	−
L-Aspartic acid	−	w	+	−	−
L-Glutamic acid	+	+	+	w	−
Glycyl L-aspartic acid	+	−	+	−	w
Glycyl L-glutamic acid	+	+	+	w	w
L-Proline	+	+	+	−	w
L-Serine	−	−	+	−	−
γ-Aminobutyric acid	−	−	+	−	−

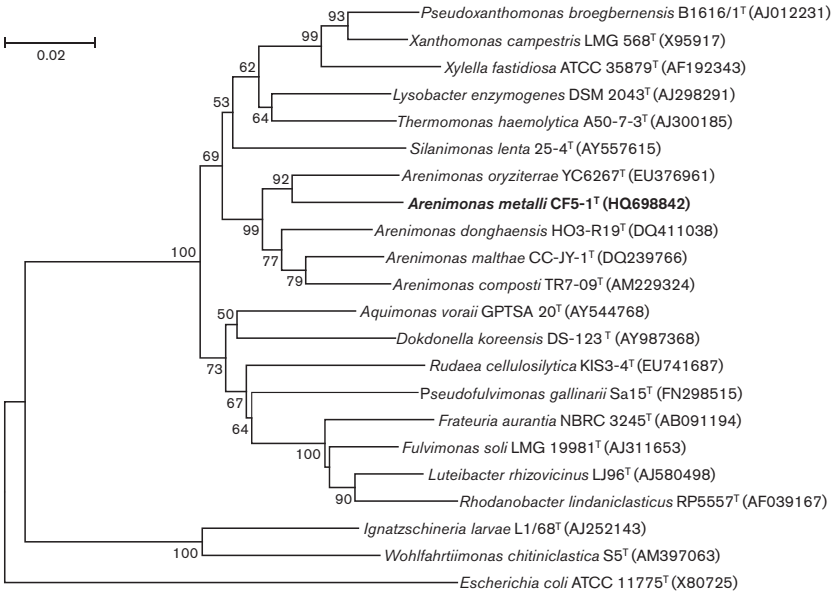

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the relationship of strain CF5-1^T to closely related taxa. Bootstrap values (>50 %) based on 1000 replications are shown at branch points. *E. coli* ATCC 11775^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

The predominant respiratory quinone was Q-8, which was the same as the four reference strains of the genus *Arenimonas* (Kwon *et al.*, 2007; Young *et al.*, 2007; Jin *et al.*, 2007; Aslam *et al.*, 2009). The DNA G+C content of strain CF5-1^T was 70.5 mol%. The major fatty acids ($\geqslant$10 %) were iso-$C_{16:0}$ (19.8 %), iso-$C_{15:0}$ (15.7 %), $C_{16:1}\omega7c$ alcohol (14.0 %) and iso-$C_{17:1}\omega9c$ (10.0 %) (Table 2). Strain CF5-1^T and the four reference strains of the genus *Arenimonas* all contained iso-$C_{16:0}$, iso-$C_{15:0}$ and iso-$C_{17:1}\omega9c$ as the main fatty acids (Table 2) (Kwon *et al.*, 2007; Young *et al.*, 2007; Jin *et al.*, 2007; Aslam *et al.*, 2009). In addition, strains CF5-1^T and *A. oryziterrae* KCTC 22247^T contained $C_{16:1}\omega7c$ alcohol, but the other three reference strains did not (this study and Aslam *et al.*, 2009). The polar lipids of strain CF5-1^T consisted of diphosphatidylglycerol (DPG), PG and PE as the major components, and small amounts of phosphatidylmonomethylethanolamine (PME) and unknown phospholipids (PL) (Fig. S3, Table 2). Among them, PG and PE were found in all the reference strains of the genus *Arenimonas*, while DPG was not found in *A. composti* KCTC 12666^T (Jin *et al.*, 2007).

On the basis of the close relationship and the distinctive phenotypic and phylogenetic differences among strain CF5-1^T and other species of the genus *Arenimonas*, it is concluded that strain CF5-1^T represents a novel species of the genus *Arenimonas*, for which the name *Arenimonas metalli* sp. nov. is proposed.

Description of *Arenimonas metalli* sp. nov.

Arenimonas metalli [me.tal′li. L. gen. n. *metalli* of the product of a mine, of a metal (as iron, etc.), referring to the source of isolation].

Cells are Gram-staining-negative, aerobic and rod-shaped (0.4–0.5 × 0.9–1.3 μm) without flagella, non-motile and non-spore-forming. Colonies on R2A agar are yellowish to creamy white, convex with entire edges, circular, smooth and 1–2 mm in diameter after incubation at 28 °C for 3 days. Grows on R2A agar, nutrient agar, 1.0 % (w/v) TSB plus 1.5 % agar, full-strength TSA and LB agar, but does not grow on MacConkey agar. The temperature range for growth is 4–37 °C (optimum, 28 °C), and the pH range for growth is pH 6–10 (optimum, pH 8.0). In R2A broth, grows in the presence of 0–1 % (w/v) NaCl (optimum, no NaCl). Positive result in tests for oxidase and catalase activities and for hydrolysis of gelatin, DNA, casein and

Table 2. Chemotaxonomic characteristics of strain CF5-1^T and related members of the genus *Arenimonas*

Strains: 1, CF5-1^T; 2, *A. malthae* CCUG 53596^T; 3, *A. oryziterrae* KCTC 22247^T; 4, *A. donghaensis* DSM 18148^T; 5, *A. composti* KCTC 12666^T. All strains contained PG and PE as major polar lipids. All fatty acids data are from this study and are the mean values of two tests. Values are percentages of total fatty acids. −, <1 % or not detected.

Characteristic	1	2	3	4	5
Fatty acid:					
iso-$C_{11:0}$	−	−	−	3.4	3.1
iso-$C_{11:0}$ 3-OH	6.0	6.0	4.5	6.9	6.3
$C_{14:0}$	−	−	2.1	−	−
iso-$C_{14:0}$	9.4	9.2	14.0	5.3	7.0
iso-$C_{15:0}$	15.7	24.2	14.9	25.9	23.1
anteiso-$C_{15:0}$	1.0	−	1.1	−	1.2
iso-$C_{15:1}$ F	−	−	−	3.1	−
iso-$C_{15:1}$ AT 5	6.4	−	3.7	−	−
$C_{16:0}$	2.8	−	6.0	2.1	−
iso-$C_{16:0}$	19.8	27.5	15.5	21.5	25.9
iso-$C_{16:1}$ G	−	2.6	−	−	−
iso-$C_{16:1}$ H*	1.0	−	−	1.3	2.5
$C_{16:1}\omega5c$	1.2	−	−	−	−
$C_{16:1}\omega11c$	1.7	−	5.5	−	−
$C_{16:1}\omega7c$ alcohol	14.0	−	10.1	−	−
iso-$C_{17:0}$	−	−	−	1.2	−
iso-$C_{17:1}\omega9c$	10.0	21.6	11.6	17.6	11.2
Summed feature 1†	−	1.5	−	1.8	8.5
Summed feature 3†	4.1	4.3	7.9	7.0	7.0
Major polar lipids‡	DPG	DPG[a]	DPG[b]	DPG[c]	PME[d]
DNA G+C content (mol%)‡	70.5	70.4[a]	65.8[b]	65.0[c]	70.8[d]

*The location of the double bond is uncertain.

†Summed features 1 and 3 comprise iso-$C_{15:1}$ H* and/or $C_{13:0}$ 3-OH, and $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH, respectively.

‡Data from: *a*, Young *et al.* (2007); *b*, Aslam *et al.* (2009); *c*, Kwon *et al.* (2007); *d*, Jin *et al.* (2007).

L-tyrosine, but negative result in tests for indole production, nitrate reduction and hydrolysis of starch, Tween 80, CM-cellulose, urea and aesculin. In API 20NE tests, shows a positive reaction only for gelatin hydrolysis, but negative reactions for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, β-galactosidase, urease, aesculin hydrolysis and assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In the GN2 MicroPlate, the following substrates are utilized: pyruvic acid methyl ester, β-hydroxybutyric acid, L-alaninamide, L-alanyl glycine, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid and L-proline. In API ZYM tests, there are positive results for the following enzyme activities: alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, trypsinase, α-chymotrypsin, acid phosphatase and naphthol-AS-Bl-phosphohydrolase, but negative results for valine arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase. Resistant to (per disc): sulfamethoxazole (75 μg), cefalotin (30 μg), lincomycin (2 μg) and oxacillin (1 μg), but sensitive to ampicillin (10 μg), erythromycin (15 μg), kanamycin (30 μg), neomycin (30 μg), penicillin (10 μg), streptomycin (10 μg), novobiocin (5 μg), chloramphenicol (30 μg), rifampicin (5 μg), amoxicillin (10 μg), vancomycin (30 μg), norfloxacin (10 μg), tetracycline (30 μg) and fourteen other types of antibiotics. Acid is not produced from L-arabinose, *N*-acetylglucosamine, propylene glycol, L-sorbose, melibiose, maltose, sucrose, trehalose, D-mannitol, D-sorbitol, inositol, melezitose, D-fructose, D-mannose, raffinose, D-ribose, D-glucose, D-galactose, lactose, L-rhamnose, D-xylose, turanose, cellobiose, adonitol and salicin. The predominant respiratory quinone is Q-8. The major fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and iso-$C_{17:1}\omega9c$. The major polar lipids are DPG, PG and PE. The MICs for Sb^{3+}, As^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Cr^{6+} and Fe^{3+} are 0.1, 0.1, 0.1, 0.3, 0.3, 0.4, 0.5 and 0.5 mM, respectively.

The type strain, CF5-1^T (=CGMCC 1.10787^T=KCTC 23460^T=CCTCC AB 2010449^T), was isolated from Hongshan Iron Mine, Daye City, Hubei province, China. The DNA G+C content of the type strain is 70.5 mol%.

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