

Actinotalea ferrariae sp. nov., isolated from an iron mine, and emended description of the genus *Actinotalea*

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A Gram-stain-positive, aerobic, non-motile, rod-shaped bacterium, designated strain CF5-4^T, was isolated from iron mining powder. 16S rRNA gene sequence analysis grouped strain CF5-4^T in a single cluster with *Actinotalea fermentans* DSM 3133^T (97.6% similarity). The major fatty acids (>5%) of strain CF5-4^T were anteiso-C_{15:0}, anteiso-C_{15:1} A, C_{16:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}. The predominant respiratory quinone was MK-10(H₄) and the genomic DNA G+C content was 74.7 mol%. The major polar lipids were diphosphatidylglycerol and one unidentified phosphoglycolipid. The peptidoglycan type of strain CF5-4^T was A4β, containing L-Orn-D-Ser-D-Asp. The cell-wall sugars were rhamnose, fucose, mannose and galactose. The results of DNA-DNA hybridization in combination with the comparison of phenotypic and phylogenetic characteristics among strain CF5-4^T and related micro-organisms revealed that the isolate represents a novel species of the genus *Actinotalea*, for which the name *Actinotalea ferrariae* sp. nov. is proposed. The type strain is CF5-4^T (=KCTC 29134^T=CCTCC AB2012198^T).

The family *Cellulomonadaceae* was originally defined by Stackebrandt & Prauser (1991) and included the genera *Actinotalea* (one species), *Cellulomonas* (21 species), *Oerskovia* (four species), *Paraoerskovia* (one species) and *Tropheryma* (one species). The genus *Actinotalea* was first described by Yi *et al.* (2007) with *Actinotalea fermentans* DSM 3133^T as the type strain. This type strain was previously isolated from dumping ground samples and identified as representing *Cellulomonas fermentans* (Bagnara *et al.*, 1985). It was reclassified in the new genus *Actinotalea* based on the major respiratory quinone MK-10(H₄) and phylogenetic analysis results (Yi *et al.*, 2007). The characteristics of *A. fermentans* were Gram-stain-positive, non-motile, facultatively anaerobic rods, containing L-Orn-D-Asp (type A4β) in the peptidoglycan, anteiso-C_{15:0}, C_{16:0}, iso-C_{16:0}, C_{14:0} and iso-C_{14:0} as the major fatty acids (>5%), MK-10(H₄) as the major isoprenoid quinone, diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) as the major polar lipids, and possessing relatively high DNA G+C content of 75.8 mol% (Bagnara *et al.*, 1985; Yi *et al.*, 2007; Shi *et al.*, 2012). The main difference between the genera *Cellulomonas* and *Actinotalea* was the major respiratory quinone [MK-9(H₄) for the

genus *Cellulomonas* and MK-10(H₄) for the genus *Actinotalea* (Stackebrandt *et al.*, 2006, Yi *et al.*, 2007; Shi *et al.*, 2012)].

Iron mining powder was collected from Hongshan Iron Mine (about 100 m underground, 30° 04' 38.77" N 114° 57' 24.07" E) of Daye City, Hubei Province, China. The pH and the total C, N, P, S and Fe concentrations were described in a previous study (Chen *et al.*, 2012). For bacterial isolation, serially diluted samples were spread on R2A (Difco) agar plates and incubated at 28 °C for 7 days. A single colony of strain CF5-4^T was picked and subcultivated. Routine cultivation was performed at 28 °C for 4 days on trypticase soy agar (TSA) and the strain was preserved at -80 °C in trypticase soy broth (TSB) supplemented with glycerol (25%, v/v).

For analyses of morphological, physiological and biochemical characteristics, strain CF5-4^T and the type strains of four close relatives, *A. fermentans* DSM 3133^T, *Cellulomonas carbonis* KCTC 19824^T, *Cellulomonas bogoriensis* DSM 16987^T and *Cellulomonas flavigena* DSM 20109^T, were cultivated on TSA agar or in TSB and incubated at 28 °C for 4 days unless otherwise mentioned. Gram staining was performed using the method described by Dussault (1955) combined with the KOH lysis method (Ryu, 1938). Cell morphology was observed using a scanning electron microscope (JSM-6390; JEOL) and a transmission electron microscope (H-7650; Hitachi). Growth at 4, 15, 20, 28, 37, 40 and 45 °C was tested on

Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Actinotalea ferrariae* CF5-4^T is HQ730135.

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

TSA for 2 weeks. Various NaCl (1–10%, w/v) and pH (4.0–11.0) ranges were assessed using TSB after incubation for 7 days. In the pH tests, the broth was adjusted to an initial pH with citrate/phosphate or Tris/HCl buffers (Breznak & Costilow, 1994). Anaerobic growth was tested on TSA with incubation in an anaerobic chamber (Mitsubishi Gas Chemical) at 28 °C for 2 weeks. Growth on nutrient agar, MacConkey agar, R2A agar and Luria–Bertani (LB) agar (all from Difco) was also investigated. Catalase activity was determined by assessing bubble production in 3% (v/v) H₂O₂, and oxidase activity was determined using 1% (w/v) tetramethyl- β -phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of gelatin, starch, casein, tyrosine, CM-cellulose, DNA, urea and Tween 80 was investigated according to Cowan & Steel (1965). Nitrate reduction was tested by the method described by Lányi (1987). H₂S, indole production, methyl red and Voges–Proskauer tests were carried out using the method described by Smibert & Krieg (1994). Acid production from various carbohydrates was determined as described by Hugh & Leifson (1953). Antibiotic-susceptibility tests were performed by spreading bacterial suspensions on TSA plates and applying filter-paper discs containing different antibiotics (Hangzhou Microbial Reagent), incubated at 28 °C for 7 days as described by Chen *et al.* (2012). Other physiological and biochemical characteristics and enzyme activities were determined using API 20 NE, API ID 32 GN and API ZYM systems according to the manufacturer's instructions (bioMérieux). The results from API ZYM test strips were observed after incubation at 28 °C for 6 h, and the API 20 NE and API ID 32 GN test results were examined after incubation at 28 °C for 48 and 72 h, respectively.

The nearly full-length 16S rRNA gene sequence was amplified as described by Fan *et al.* (2008) and compared with the sequences available in EzTaxon-e server (<http://eztaxon-e.ezbiocloud.net/>) (Kim *et al.*, 2012) and NCBI GenBank database. Multiple alignments of sequences were carried out using CLUSTAL X (Thompson *et al.*, 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) trees were reconstructed using MEGA 4.0 (Tamura *et al.*, 2007), and a maximum-likelihood tree was generated using the PHYML online web server (Guindon *et al.*, 2005). To obtain the confidence level of the branches, bootstrap values were calculated based on 1000 replications (Felsenstein, 1985).

For whole-cell fatty acid analysis, strain CF5-4^T and *A. fermentans* DSM 3133^T, *C. carbonis* KCTC 19824^T and *C. bogoriensis* DSM 16987^T were grown in TSB at 28 °C until they reached the late exponential phase and 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). Polar lipids were determined by two-dimensional TLC as described by Tindall (1990). Peptidoglycan and sugars in the whole cell-wall were analysed by the method of Schumann (2011).

The DNA G+C content was determined by HPLC as described by Mesbah *et al.* (1989). The respiratory quinones were extracted and identified by HPLC as described by Minnikin *et al.* (1984). Levels of DNA–DNA relatedness were determined using the thermal denaturation and renaturation method of Huss *et al.* (1983).

Cells of strain CF5-4^T were Gram-stain-positive, aerobic, non-motile rods. Colonies were yellow, convex, circular, smooth and non-transparent after 4 days incubation on TSA at 28 °C. The strain grew on TSA, nutrient agar, R2A agar and LB agar, but did not grow on MacConkey agar. Detailed results of the polyphasic characterization of strain CF5-4^T are given in the species description. A scanning electron micrograph and a transmission electron micrograph showing the general morphologies of strain CF5-4^T are available in IJSEM Online (Fig. S1). The main differential phenotypic characteristics between strain CF5-4^T and the four reference strains are shown in Table 1.

The 1483 bp 16S rRNA gene sequence of strain CF5-4^T was analysed to determine its phylogenetic position. Strain CF5-4^T showed high sequence similarities to species of the genera *Cellulomonas* and *Actinotalea*. Highest 16S rRNA gene sequence similarities were to *C. carbonis* T26^T (=KCTC 19824^T) (98.4%), followed by *A. fermentans* DSM 3133^T (97.6%) and *C. bogoriensis* 69B4^T (=DSM 16987^T) (96.6%). The 16S rRNA gene sequence similarity between strain CF5-4^T and *C. flavigena* DSM 20109^T (type species of the genus *Cellulomonas*) was 94.6%. These four type strains were therefore used as reference strains. A phylogenetic tree reconstructed using the neighbour-joining algorithm grouped strain CF5-4^T in a cluster with *A. fermentans* DSM 3133^T. Although strain CF5-4^T showed highest similarity to *C. carbonis* T26^T, all species of the genus *Cellulomonas*, including *C. carbonis* T26^T, fell into other nearby positions (Fig. 1). The maximum-parsimony tree and the maximum-likelihood trees (Fig. S2) also supported the phylogenetic position obtained with the neighbour-joining method.

The major cellular fatty acids of strain CF5-4^T (>5%) were anteiso-C_{15:0} (38.4%), anteiso-C_{15:1} A (19.2%), C_{16:0} (9.5%), iso-C_{16:0} (6.9%), iso-C_{15:0} (7.7%) and anteiso-C_{17:0} (7.1%) (Table 2). Strain CF5-4^T exhibited similar polar lipids of DPG and PG as the four reference type strains. It also had one unidentified phosphoglycolipid (PGL), one unidentified phospholipid (PL), one unidentified glycolipid (GL) and phosphatidylinositol mannoside (PIM) (Fig. S3). The peptidoglycan type of CF5-4^T was A4 β , containing L-Orn–D-Ser–L-Asp. The cell-wall sugars were rhamnose, fucose, mannose and galactose. These results were similar to those for the four reference strains. The DNA G+C content of strain CF5-4^T was 74.7 mol%. The major respiratory quinone was MK-10(H₄), which was the same as *A. fermentans*, but was different from members of the genus *Cellulomonas*, which contained MK-9(H₄) as the major respiratory quinone (Yi *et al.*, 2007; Abt *et al.*,

Table 1. Differential phenotypic characteristics between strain CF5-4^T and the type strains of closely related *Actinotalea* and *Cellulomonas* species

Strains: 1, CF5-4^T; 2, *A. fermentans* DSM 3133^T; 3, *C. carbonis* KCTC 19824^T; 4, *C. bogoriensis* DSM 16987^T; 5, *C. flavigena* DSM 20109^T. Data are from this study, except for the peptidoglycan, cell-wall sugars, DNA G + C content and polar lipids of the reference strains. +, Positive; -, negative; W, weakly positive; ND, no data; Y, yellow; WH, white; YW, yellow-white; D-Glu, D-glutamic acid; L-Orn, L-ornithine; D-Ser, D-serine; D-Asp, D-aspartate.

Characteristic	1	2	3	4	5
Motility	-	-	+	+	-
Colony colour	Y	WH	YW	Y	Y
NaCl range (% w/v)	0-7	0-5	0-8	0-8	0-5
Nitrate reduction	+	+	+	-	+
H ₂ S production	-	-	+	-	-
Catalase	+	-	+	+	+
DNase	-	+	-	+	-
Utilization of:					
D-Ribose	+	-	-	-	+
L-Rhamnose	-	-	-	+	-
Melibiose	-	-	+	-	-
Sorbitol	-	-	+	-	-
Mannitol	-	+	+‡	-	-
L-Arabinose	+	+	+	-	+
Acetate	+	+	-	-	+
Fermentation of:					
D-Galactose	+	+	+	-	+
Lactose	-	-	+	-	-
D-Sorbitol	-	+	+	-	w
N-Acetylglucosamine	-	-	+	-	-
D-Mannitol	+	-	-	-	-
Peptidoglycan	L-Orn-D-Ser-D-Asp	L-Orn-D-Asp ^a §	L-Orn-D-Glu ^b	L-Orn-D-Asp ^c	L-Orn-D-Asp ^d
Cell-wall sugars*	Rha	Rha	Rha	ND	Rha
	Fuc	Glc	Gal		GlcN
	Man	Rib ^e	Xyl		Rib
	Gal		Ino ^b		Man ^d
DNA G + C content (mol%)	74.7	75.8 ^e	74.4 ^b	71.5 ^c	73.8 ^d
Major polar lipids†	DPG	DPG	DPG	DPG	DPG
	PGL	PG ^b	PG ^b	PG ^b	PI ^d

*Fuc, fucose; Gal, galactose; Glc, glucose; GlcN, glucosamine; Ino, inositol; Man, mannose; Rha, rhamnose; Rib, ribose; Xyl, xylose.

†DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PGL, unidentified phosphoglycolipid.

‡Data different from the previous study (Shi *et al.*, 2012).

§Data are taken from: a, Yi *et al.* (2007); b, Shi *et al.* (2012); c, Jones *et al.* (2005); d, Abt *et al.* (2010); e, Bagnara *et al.* (1985).

2010; Shi *et al.*, 2012). The results of DNA-DNA hybridization showed 55.6 % relatedness between strain CF5-4^T and *A. fermentans* DSM 3133^T and 52.9 % relatedness between strain CF5-4^T and *C. carbonis* KCTC 19824^T, suggesting that strain CF5-4^T should be classified as representing a novel species, in view of the recommendations of Wayne *et al.* (1987) for species delineation. Strain CF5-4^T could be differentiated from species of the genus *Cellulomonas* on the basis of the respiratory quinone [MK-10(H₄) versus MK-9(H₄)], phylogenetic classification and DNA-DNA relatedness values.

Based on analyses of its polyphasic properties, it is concluded that strain CF5-4^T represents a novel species

of the genus *Actinotalea*, for which the name *Actinotalea ferrariae* sp. nov. is proposed.

Emended description of the genus *Actinotalea* (Yi *et al.*, 2007)

The description is as given by Yi *et al.* (2007) with the following amendments. The major fatty acids (>5 %) are anteiso-C_{15:0}, C_{16:0} and iso-C_{16:0}. The major polar lipid is DPG. The peptidoglycan type is A4β, containing L-Orn-D-Asp or L-Orn-D-Ser-D-Asp. The major cell-wall sugar is rhamnose; fucose, mannose, galactose, glucose and ribose are also present. The DNA G + C content is 74.7–75.8 mol%. The type species is *Actinotalea fermentans*.

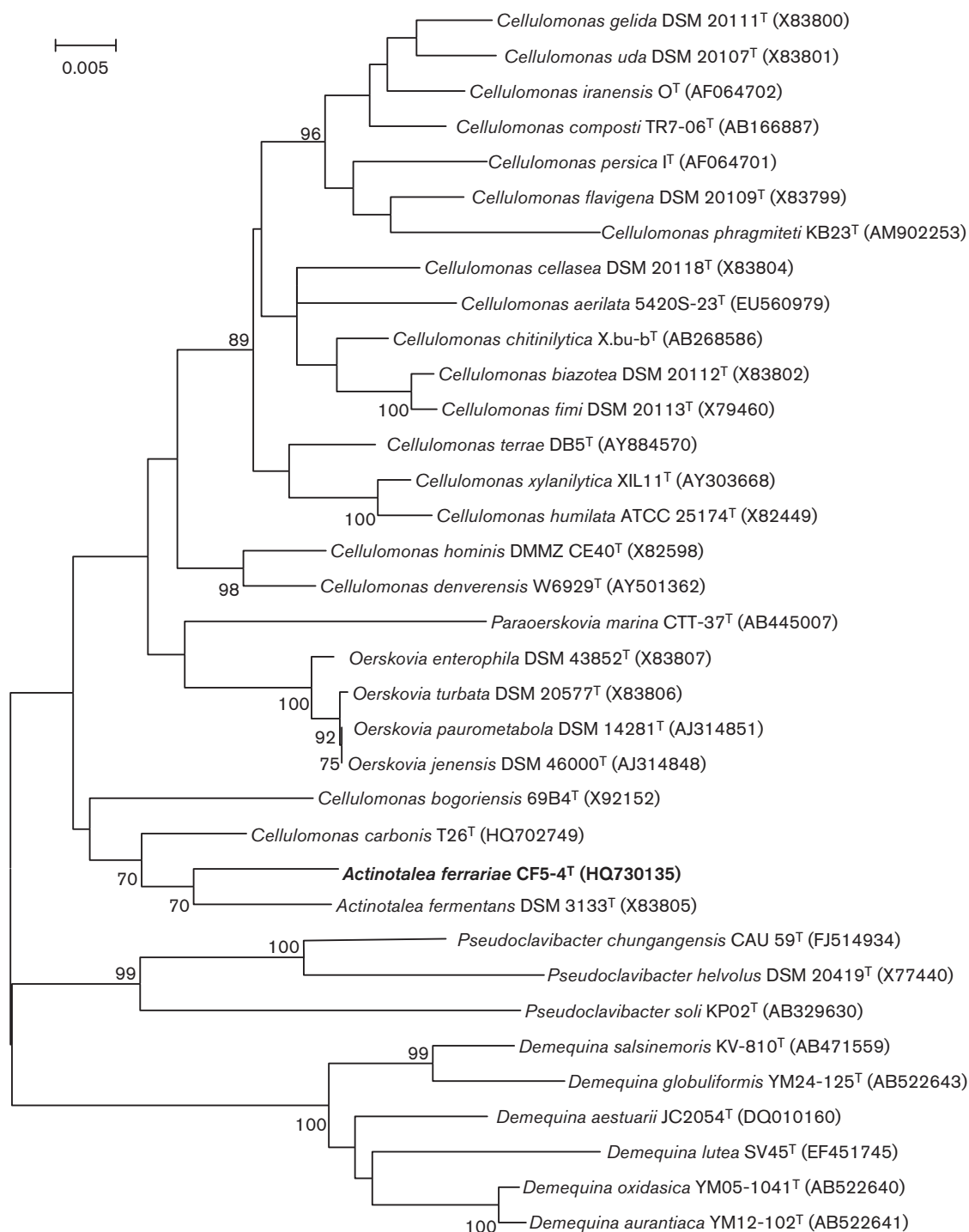


Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain CF5-4^T among its closest neighbours. Bootstrap values ($\geq 70\%$) based on 1000 replications are shown at branch nodes. Bar, 5 substitutions per 1000 nt.

Description of *Actinotalea ferrariae* sp. nov.

Actinotalea ferrariae (fer.ra.ri'a.e. L. gen. n. *ferrariae* of an iron mine).

Cells are Gram-stain-positive, aerobic, non-motile and rod-shaped (0.4–0.5 × 1.0–1.3 μm). Colonies are yellow,

convex, circular, smooth and non-transparent after incubation on TSA at 28 °C for 7 days. Grows on full-strength TSA, nutrient agar, R2A agar and LB agar, but not on MacConkey agar. Temperature range for growth is 4–40 °C, and optimal temperature is 28 °C. Growth occurs with NaCl concentrations in the range 0–7% (optimum,

Table 2. Cellular fatty acid compositions (%) of strain CF5-4^T and the type strains of closely related *Actinotalea* and *Cellulomonas* species

Strains: 1, CF5-4^T; 2, *A. fermentans* DSM 3133^T; 3, *C. carbonis* KCTC 19824^T; 4, *C. bogoriensis* DSM 16987^T; 5, *C. flavigena* DSM 20109^T. Data are from this study, except for strain 5 (taken from Shi *et al.*, 2012). –, <1% or not detected.

Fatty acid	1	2	3	4	5
C _{13:0}	–	–	–	–	1.8
anteiso-C _{13:0}	–	–	–	1.4	–
C _{14:0}	3.0	18.5	6.0	9.7	12.1
iso-C _{14:0}	3.0	12.1	2.8	1.4	3.4
anteiso-C _{15:1} A	19.2	–	11.8	2.2	21.5
C _{15:0}	–	–	1.0	1.4	6.8
iso-C _{15:0}	7.7	3.5	3.4	3.6	2.6
anteiso-C _{15:0}	38.4	33.7	38.8	46.5	34.0
C _{16:0}	9.5	18.6	16.1	24.8	12.6
iso-C _{16:0}	6.9	12.5	6.4	3.0	1.5
iso-C _{17:0}	1.8	–	–	–	–
anteiso-C _{17:0}	7.1	–	8.6	3.8	1.1
C _{18:0}	–	–	1.4	–	–

3%) and pH 6.0–8.0 (optimum, pH 7.0). Catalase-positive and oxidase-negative. Positive for β-galactosidase activity but negative for arginine dihydrolase and urease activities. Hydrolyses CM-cellulose, starch, gelatin and aesculin, but not DNA, casein, tyrosine or Tween 80. Does not produce indole or H₂S, but NH₃ is produced from peptone. Positive for nitrate reduction but negative for methyl red test and Voges–Proskauer reaction. Assimilates D-glucose, D-ribose, L-arabinose, maltose, sucrose, glycogen, salicin, xylose and sodium acetate, but not L-rhamnose, N-acetylglucosamine, inositol, itaconic acid, suberate, sodium malonate, lactate, propionate, L-alanine, potassium 5-ketoglucuronate, 3-hydroxybenzoate, L-serine, mannitol, melibiose, L-fucose, D-sorbitol, propionate, caprate, valerate, trisodium citrate, L-histidine, potassium 2-ketoglucuronate, 3-hydroxybutyrate, 4-hydroxybenzoate or L-proline (API 20NE and API ID 32 GN). In API ZYM tests, positive for esterase (C4), N-acetyl-β-glucosaminidase, acid phosphatase, α-galactosidase, naphthol-AS-BI-phosphohydrolase, leucine arylamidase, alkaline phosphatase, esterase lipase (C8) and valine arylamidase activities, but negative for β-galactosidase, α-glucosidase, β-glucosidase, lipase (C14), trypsin, α-chymotrypsin, cystine arylamidase, β-fucosidase, β-glucuronidase and α-mannosidase activities. Acid is produced from D-glucose, cellobiose, D-fructose, D-mannose, D-galactose, maltose, sucrose, D-xylose, L-arabinose and D-mannitol, but not from lactose, D-sorbitol, N-acetylglucosamine, D-arabinose, inositol, L-sorbose or L-rhamnose. Sensitive to ampicillin (10 μg), carbenicillin (100 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), erythromycin (15 μg), furazolidone (15 μg), norfloxacin (10 μg), ofloxacin (5 μg), penicillin (10 μg), piperacillin (100 μg), ceftriaxone (100 μg), vancomycin (30 μg), doxycycline

(30 μg), kanamycin (30 μg), minocycline (30 μg), cephalosporin IV (30 μg), cephalosporin VI (30 μg), medemycin (30 μg), amikacin (30 μg), cefoperazone (30 μg), tetracycline (30 μg), neomycin (10 μg), oxacillin (1 μg), polymyxin B (30 μg), sulfamethoxazole (75 μg), cephalosporin V (30 μg) and ceftazidime (30 μg), but resistant to cefuroxime (30 μg) and gentamicin (10 μg). The peptidoglycan type is A4β, containing L-Orn–D-Ser–L-Asp. The cell-wall sugars are rhamnose, fucose, mannose and galactose. Major fatty acids (>5%) are anteiso-C_{15:0}, anteiso-C_{15:1} A, C_{16:0}, iso-C_{16:0}, iso-C_{15:0} and anteiso-C_{17:0}. The major respiratory quinone is MK-10(H₄). Major polar lipids are DPG and one unidentified phosphoglycolipid.

The type strain, CF5-4^T (=KCTC 29134^T=CCTCC AB2012198^T), was isolated from iron mining powder of Hongshan Iron Mine of Daye City, Hubei Province, China. The DNA G + C content of the type strain is 74.7 mol%.

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