Fodinicurvata sediminis gen. nov., sp. nov. and *Fodinicurvata fenggangensis* sp. nov., poly-β-hydroxybutyrate-producing bacteria in the family *Rhodospirillaceae*

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Two Gram-negatively stained, facultatively anaerobic, non-motile, vibrioid and rod-shaped, chemoheterotrophic bacterial strains, designated YIM D82^T and YIM D812^T, were isolated from a salt mine in Yunnan, south-west China. DNA–DNA hybridization, genomic DNA G+C content and phylogenetic analyses based on 16S rRNA gene sequences divided the two isolates into two distinct genospecies that were also clearly differentiated by fatty acid profiles, carbon source utilization patterns, antibiotic susceptibility and biochemical characteristics. The two isolates grew in the presence of 1.5–20 % NaCl, and optimally at 28 °C and pH 7.5. The genomic DNA G+C contents of strains YIM D82^T and YIM D812^T were 61.5 and 62.3 mol%, respectively. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strains YIM D82^T and YIM D812^T were members of the family *Rhodospirillaceae* and showed 90.5–90.6 % and 90.1–90.2 % similarities with their closest relatives, *Rhodovibrio sodomensis* and *Rhodovibrio salinarum*, respectively. Differential phenotypic and genotypic characteristics of the two isolates from recognized genera showed that the two strains should be classified as representing a new genus and two novel species for which the names *Fodinicurvata sediminis* gen. nov., sp. nov. (type strain YIM D82^T=DSM 21159^T=KCTC 22351^T) and *Fodinicurvata fenggangensis* sp. nov. (type strain YIM D812^T=CCTCC AA 208037^T=DSM 21160^T) are proposed.

The family *Rhodospirillaceae*, belonging to the order *Rhodospirillales* (Pfennig & Trüper, 1971) of the class *Alphaproteobacteria*, comprises 21 genera (http://www.bacterio.cict.fr). During the course of a study of the microbial diversity of the Fenggang salt mine in Yunnan, south-west China, two cream–white-pigmented bacterial strains, designated YIM D82^T and YIM D812^T, were isolated. The aim of the present study was to determine the exact taxonomic positions of strains YIM D82^T and YIM D812^T by using a polyphasic approach that included the analysis of phenotypic properties, detailed phylogenetic analysis based on 16S rRNA gene sequences and DNA–DNA relatedness.

Strains YIM D82^T and YIM D812^T were isolated from a sediment sample collected from the salt mine by using a standard dilution-plating technique at 28 °C on Difco marine agar 2216 (MA; pH 7.2), supplemented with 3 % (w/v) NaCl. Pure cultures were maintained on nutrient agar (NA; Difco) supplemented with 5 % NaCl, and stored as 20 % (v/v) glycerol suspensions at −80 °C.

Gram staining was performed using the method of Magee *et al.* (1975) with crystal violet (60 s), iodine mordant (60 s), 95 % ethanol (5–10 s) and safranin counterstain (60 s), and with the 3 % KOH lysis test (Gregersen, 1978) as a supplementary test to Gram staining. Cellular morphology and motility were examined by using light microscopy (model BH 2; Olympus). Growth at various

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Abbreviation: PHB, poly-β-hydroxybutyrate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM D82^T and YIM D812^T are FJ357426 and FJ357427, respectively.

Two-dimensional thin-layer chromatograms of the polar lipids of strains YIM D82^T and YIM D812^T and a table showing the cellular fatty acid contents of strains YIM D82^T and YIM D812^T are available as supplementary material with the online version of this paper.

concentrations of salt was determined on NA supplemented with NaCl at 0, 0.5, 1, 1.5, 2, 2.5, 3, 5, 7, 10, 12, 15, 20, 25 and 30 % (w/v). Growth at various pH values (4–11, in increments of 0.5 pH units) and temperatures (4, 10, 15, 20, 25, 28, 37, 42, 45, 50, 55 and 60 °C) were determined on the maintenance medium (NA supplemented with 5 % NaCl). Growth under anaerobic conditions was determined after incubation in an anaerobic chamber (GasPak Anaerobic system; BBL) on NA supplemented with 5 % NaCl with or without nitrate. Accumulation of poly-β-hydroxybutyrate (PHB) was observed by using both negatively stained electron microscopy and Sudan black staining (Smibert & Krieg, 1994) under a light microscope. Bacteriochlorophyll *a* was analysed spectrophotometrically according to the procedure of Cohen-Bazire *et al.* (1957) following the recommendations of Allgaier *et al.* (2003). Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. L-Phenylalanine deamination was examined using the method of Richard & Kiredjian (1995). Citrate utilization was tested on Simmons' citrate agar (Sigma). H_2S production was determined on Kligler iron agar (Difco). Methyl red and Voges–Proskauer tests were performed as described by Smibert & Krieg (1994). Nitrate reduction, hydrolysis of aesculin and gelatin, acid production from glucose, indole production, arginine dihydrolase, urease and β-galactosidase were tested using an API 20NE kit (bioMérieux), according to the manufacturer's instructions. Other enzyme activities were assayed by using an API ZYM kit (bioMérieux), except that the bacterial suspensions were prepared in autoclaved 5 % NaCl solution. Carbon utilization was tested using artificial seawater medium (Cho & Giovannoni, 2006) as the basal medium with each carbon source at a final concentration of 0.5 % (w/v or v/v). Antibiotic resistance was determined with the disc diffusion method using commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were estimated according to the formation of the inhibition zone.

Isoprenoid quinones were extracted by using the method of Collins *et al.* (1977) and analysed by HPLC as described by Tamaoka *et al.* (1983). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and were identified by using two-dimensional TLC after spraying with the appropriate detection reagents (Collins & Jones, 1980). The presence of phosphatidylcholine was identified by spraying with Dragendorff reagent (Sigma). Biomass for quantitative fatty acid analysis of the two strains was prepared by scraping growth from NA supplemented with 5 % NaCl that had been incubated for 5 days at 28 °C. Analysis of the cellular fatty acid profiles followed the method described by Sasser (1990) using the Microbial Identification System (MIDI). The G+C content of the genomic DNA was determined by using HPLC according to Mesbah *et al.* (1989), after extraction of DNA using the method of Cui *et al.* (2001). The genomic DNA of *Escherichia coli* DH5α was used as a standard.

The 16S rRNA gene was amplified and sequenced as described by Cui *et al.* (2001). The sequence was compared to those available in GenBank using BLAST (Altschul *et al.*, 1990). Alignments and similarities were obtained using CLUSTAL_X. Phylogenetic analyses were carried out using MEGA3 (Kumar *et al.*, 2004). Distances (corrected according to the Kimura two-parameter model; Kimura, 1980) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). A maximum-likelihood (Felsenstein, 1981) tree (not shown) was generated using the treeing algorithm contained in the PHYLIP package (Felsenstein, 1993). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1000 resamplings (Felsenstein, 1985).

Strains YIM D82^T and YIM D812^T were both facultatively anaerobic, Gram-negative and non-motile. Cells were vibrioid and rod-shaped. PHB granules were detected in the two strains by using both negatively stained electron microscopy and Sudan black staining. Neither flagella nor endospores were observed. Colonies were cream–white, circular, convex and opaque with irregular margins after growth on NA supplemented with 5 % at 28 °C for 5 days. Growth occurred under anaerobic conditions. The temperature range for growth was 15–42 °C (optimum, 28 °C) and the pH range for growth was 6.5–8.5 (optimum, 7.5). Growth occurred at NaCl concentrations of 1.5–20 % (w/v) (optimum, 5 %). The two strains were catalase- and oxidase-positive. They were negative for Methyl red and Voges–Proskauer reactions. The two strains did not produce H_2S or L-phenylalanine deaminase. Biochemical tests for nitrate reduction, arginine dihydrolase and urease were positive. Hydrolysis of aesculin and gelatin, indole production, glucose acidification, and phenylalanine deaminase and β-galactosidase were negative. Acetone/methanol-extractable pigments and bacteriochlorophyll *a* were not produced. Therefore, the energy metabolism of the two strains appeared to be exclusively non-photosynthetic chemoheterotrophy. Major characteristics that differentiate the two strains are given in Table 1. Carbon source utilization patterns and antibiotic susceptibility are given in the species descriptions.

The major cellular fatty acids of strain YIM D82^T comprised $C_{18:1}\omega7c$ (48.6 %), $C_{18:1}$ 2-OH (12.2 %) and $C_{16:0}$ (11.8 %), and those of strain YIM D812^T comprised $C_{18:1}\omega7c$ (46.6 %), $C_{18:1}$ 2-OH (14.7 %) and $C_{16:0}$ (10.6 %) (see Supplementary Table S1, available in IJSEM Online). The fatty acid $C_{18:1}\omega7c$ was commonly found as a major component in both strains YIM D82^T and YIM D812^T, which is a feature shared by members of the class *Alphaproteobacteria* (Labrenz *et al.*, 2000). However, moderate amounts of $C_{18:1}$ 2-OH (12.2–14.7 %) and $C_{19:0}$ cyclo $\omega8c$ (7.9–7.4 %) were found in strains YIM D82^T and YIM D812^T, but were not found at significant levels in the genus *Rhodovibrio*. In addition, the presence of fatty acids iso-$C_{15:0}$ G, $C_{18:1}\omega9c$ and $C_{20:2}$ $\omega6,9c$ clearly differentiated the two novel isolates from the genera *Rhodovibrio*, *Azospirillum*, *Tistrella*, *Inquilinus* and

Table 1. Characteristics that differentiate strains YIM D82^T and YIM D812^T from other phylogenetically related genera in the family *Rhodospirillaceae*

Taxa: 1, strain YIM D82^T (*Fodinicurvata sediminus* gen. nov., sp. nov.); 2, strain YIM D812^T (*Fodinicurvata fenggangensis* sp. nov.); 3, *Rhodovibrio* (data from Nissen & Dundas, 1984; Mack *et al.*, 1993; Imhoff *et al.*, 1998; Garrity *et al.*, 2005); 4, *Azospirillum* (Tarrand *et al.*, 1978; Reinhold *et al.*, 1987; Khammas *et al.*, 1989; Sly & Stackebrandt, 1999; Eckert *et al.*, 2001; Xie & Yokota, 2005; Peng *et al.*, 2006; Mehnaz *et al.*, 2007a, b; Young *et al.*, 2008); 5, *Rhodocista* (Favinger *et al.*, 1989; Kawasaki *et al.*, 1992; Imhoff *et al.*, 1998; Zhang *et al.*, 2003); 6, *Defluviicoccus* (Maszenan *et al.*, 2005); 7, Tistrella (Shi *et al.*, 2002); 8, *Skermanella* (Sly & Stackebrandt, 1999); 9, *Inquilinus* (Coenye *et al.*, 2002); 10, *Thalassobaculum* (Zhang *et al.*, 2008). +, Positive; W, weakly positive; −, negative; V, variable; NA, not available. All strains are Gram-negative and catalase-positive (data for catalase were not available for the genera *Rhodocista* and *Rhodovibrio*).

Characteristic	1	2	3	4	5	6	7	8	9	10
Habitat	Deposit of salt mine	Deposit of salt mine	Seawater, ponds of solar salt	Soil, root, fresh water	Freshwater, wastewater	Sludge	Wastewater	Lake water	Cystic fibrosis patients	Coastal seawater
Colony colour	Cream–white	Cream–white	Pink, red	Pink, white	Red, pink	Beige	NA	Apricot	Pink	Cream–yellow
Cell size (width × length; μm)	0.3–0.5 × 0.7–1.5	0.2–0.4 × 0.5–1.3	0.6–0.9 × 1.0–3.5	0.6–0.9 × 2–30	1–2 × 3.0	1.5–4.5	0.7–1.0 (width)	1–1.5 × 2–3	NA	0.3-0.5 × 1.3-1.5
Cell shape	Rod and vibrioid	Rod and vibrioid	Vibrioid, spiral	Plump, vibrioid straight rod	Vibrioid, spiral	Coccus	Rod	Rod	Rod	Slightly curved and straight rod
Flagella*	−	−	MP, BP	V	MP	−	MP	MP	NA	MP
Temperature range (°C)	15–42	15–42	20–47	4–41	25–47	20–30	20–40	10–37	25–42	10–35
pH range	6.5–8.5	6.5–8.5	7–8	5–8.5	5.7–8	5–8.5	5–9	NA	NA	7–9
NaCl/salt tolerance (%, w/v)	1.5–20	1.5–20	3–24	<5	NA	NA	<1	<5	<6	1–10
Poly-β-hydroxybutyrate	+	+	+	V	+	+	+	+	NA	+
Bacteriochlorophyll *a*	−	−	+	−	+	NA	−	−	NA	−
Oxidase	+	+	NA	+	NA	−	+	+	V	+
Gelatinase	+	+	NA	V	NA	W	+	−	V	+
Utilization of carbon sources										
L-Arabinose	+	−	NA	V	NA	+	+	+	−	+
D-Glucose	+	+	−	V	−	+	NA	+	−	−
Citrate	+	+	−	V	−	NA	NA	+	−	−
myo-Inositol	−	+	NA	V	NA	NA	NA	−	−	−
D-Mannitol	+	−	−	V	−	NA	+	+	−	−
L-Rhamnose	−	−	NA	V	NA	NA	NA	+	−	−
D-Ribose	−	−	NA	V	NA	NA	NA	+	NA	+
Sucrose	+	−	−	V	−	NA	NA	NA	−	+
Major quinone	Q-10	Q-10	Q-10, MK-10	Q-10	Q-9	NA	Q-10	Q-10	NA	Q-10
DNA G+C content (mol%)	61.5	62.3	66.2–68.1	64–71	68.3–69.9	66	67.5	67.2	70.9	68.0

*BP, Bipolar; MP, monopolar.

Thalassobaculum. Therefore, the fatty acid profiles of strains YIM D82^T and YIM D812^T differed distinctly from those of related genera in the family *Rhodospirillaceae*. Furthermore, strains YIM D82^T and YIM D812^T could be clearly differentiated by means of the presence or absence of $C_{13:0}$ 2-OH, $C_{14:1}\omega5c$, anteiso-$C_{15:0}$, $C_{16:0}$ 2-OH and $C_{17:1}\omega7c$. The isoprenoid quinone in strains YIM D82^T and YIM D812^T was ubiquinone 10 (Q-10), which was also found in the genera *Azospirillum*, *Tistrella*, *Skermanella* and *Thalassobaculum*. However, MK-10 and Q-9 were also found, respectively, in the genera *Rhodovibrio* and *Rhodocista* (Table 1). The polar lipids consisted of diphosphatidylglycerol, phosphatidylmethylethanolamine and phosphatidylcholine, except that strain YIM D812^T also contained phosphatidylinositol and three unknown phospholipids, and strain YIM D82^T contained one unknown phospholipid (see Supplementary Fig. S1, available in IJSEM Online). The G+C contents of the genomic DNA of strains YIM D82^T and YIM D812^T were 61.5 and 62.3 mol%, respectively.

Phylogenetic analysis of almost-complete 16S rRNA gene sequences of strains YIM D82^T and YIM D812^T revealed that they formed a distinct lineage within the family *Rhodospirillaceae* (Fig. 1). The similarity between the 16S rRNA gene sequences of the two strains was 98.2 %. Species of the genus *Rhodovibrio* were found to be the nearest phylogenetic neighbours; this relationship was supported by a high bootstrap value (92 %) and also by the other tree-making algorithm used. The levels of 16S rRNA gene sequence similarities between strain YIM D82^T and the type strains of *Rhodovibrio sodomensis* and *Rhodovibrio salinarum* were 90.6 and 90.5 %, respectively, and 85.0–89.5 % to the other type species of the family *Rhodospirillaceae*. The levels of 16S rRNA gene sequence similarities between strain YIM D812^T and the type strains of *R. sodomensis* and *R. salinarum* were 90.2 and 90.1 %, respectively, and 84.3–88.1 % to the other type species of the family *Rhodospirillaceae*. The low sequence similarity values between strains YIM D82^T and YIM D812^T and the type species of genera of the family *Rhodospirillaceae* demonstrated that the two strains represent a distinct genus in the family *Rhodospirillaceae*.

DNA–DNA hybridization was performed using the photobiotin-labelling method of Ezaki *et al.* (1989), with a multiwell plate reader (CytoFluor; PerSeptive Biosystems). The DNA–DNA relatedness between strains YIM D82^T and YIM D812^T was 27.5 %. Therefore, strains YIM D82^T and YIM D812^T should be considered as representing two separate species.

The data obtained based on the polyphasic approach used, such as fatty acid profiles, quinone and 16S rRNA gene phylogenetic analyses, demonstrated conclusively that strains YIM D82^T and YIM D812^T should be recognized as representing a novel genus within the family *Rhodospirillaceae*. In addition, the DNA–DNA hybridization values, polar lipid patterns and differences in phenotypic traits indicated that the two strains represent two novel species in the new genus, for which the names *Fodinicurvata sediminis* gen. nov., sp. nov. and *Fodinicurvata fenggangensis* sp. nov. are proposed.

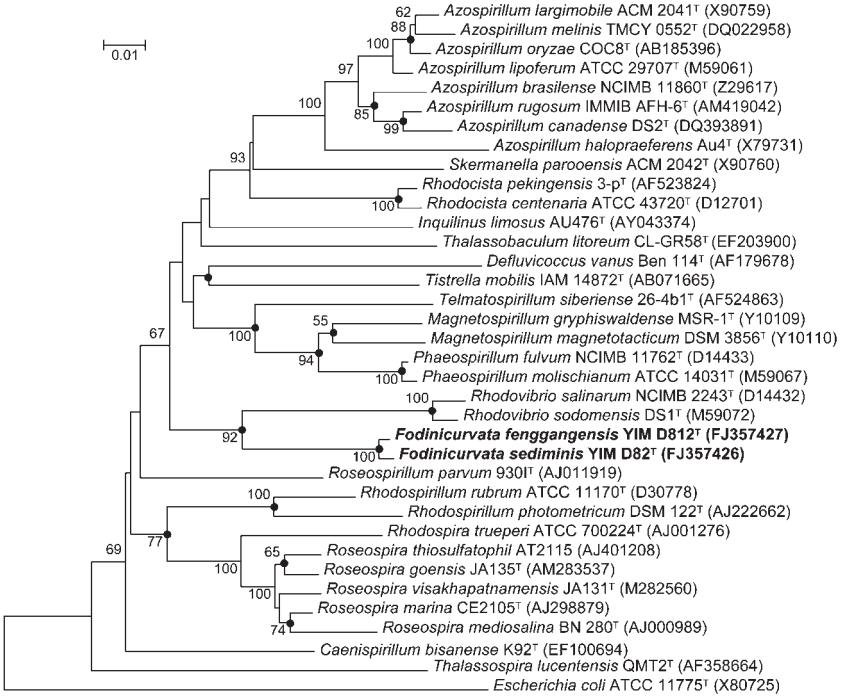

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strains YIM D82^T and YIM D812^T, and representatives of the family *Rhodospirillaceae*. Bootstrap percentages (based on 1000 replications) >50 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood method. Bar, 0.01 substitutions per nucleotide position.

Description of *Fodinicurvata* gen. nov.

Fodinicurvata (Fo.di.ni.cur.va′ta. L. fem. n. *fodina* mine; L. adj. *curvatus -a -um* curved; N.L. fem. n. *Fodinicurvata* curved-shaped bacterium isolated from a mine).

Cells are Gram-negative, facultatively anaerobic, non-motile, vibrioid and rod-shaped. Catalase- and oxidase-positive. Bacteriochlorophyll *a* is not detected. Accumulate PHB granules. Nitrate is reduced. The predominant polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine and phosphatidylcholine. Phosphatidylinositol is variable among species. The DNA G+C content is 61.5–62.3 mol%. Member of the family *Rhodospirillaceae*. The type species is *Fodinicurvata sediminis*.

Description of *Fodinicurvata sediminis* sp. nov.

Fodinicurvata sediminis (sed.i.min′is. L. gen. n. *sediminis* of sediment).

Exhibits the following properties in addition to those given in the genus description. Colonies are cream–white, smooth, circular, convex and opaque, with slightly irregular margins. Cells are approximately 0.3–0.5 μm wide and 0.7–1.5 μm long. Temperature range for growth is 15–42 °C (optimum, 28 °C). pH range for growth is 6.5–8.5 (optimum, 7.5). Grows at NaCl concentrations of 1.5–20 % (w/v). Positive for urease, arginine dihydrolase and nitrate reduction, and negative for hydrolysis of gelatin and aesculin, indole production, glucose acidification, L-phenylalanine deaminase and β-galactosidase. Utilizes L-arabinose, α-cyclodextrin, D-glucose, maltose, D-mannitol, D-sorbitol, sucrose, D-xylose, ethanol, glycerol, acetate, citrate, L-asparagine, L-aspartic acid, L-glutamic acid and L-proline as sole carbon sources, but not adonitol, amylum, D-cellulose, β-cyclodextrin, dextrin, L-fructose, D-galactose, *myo*-inositol, D-lactose, D-mannose, melibiose, methanol, raffinose, L-rhamnose, D-ribose, trehalose, glycine, L-histidine, L-ornithine, L-phenylalanine or D-serine. With the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase and trypsin, weakly positive for α-chymotrypsin and naphthol-AS-BI-phosphohydrolase and negative for acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sensitive to (μg per disc, unless indicated otherwise): ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), penicillin (10 U) and streptomycin (10), and resistant to amikacin (30), gentamicin (10), nalidixic acid (30) and norfloxacin (10). Polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine and one unknown phospholipid. Major fatty acids are $C_{18:1}\omega7c$, $C_{18:1}$ 2-OH and $C_{16:0}$. The isoprenoid quinone is Q-10. The DNA G+C content of the type strain is 61.5 mol%.

The type strain, YIM D82^T (=DSM 21159^T=KCTC 22351^T), was isolated from the Fenggang salt mine in Yunnan, south-west China.

Description of *Fodinicurvata fenggangensis* sp. nov.

Fodinicurvata fenggangensis (feng.gang.en′sis. N.L. fem. adj. *fenggangensis* the locality of the salt mine from which the organism was isolated).

Exhibits the following properties in addition to those given in the genus description. Colonies are cream–white, smooth, circular, convex and opaque with slightly irregular margins. Cells are approximately 0.2–0.5 μm wide and 0.5–1.3 μm long. Temperature range for growth is 15–42 °C (optimum, 28 °C). pH range for growth is 6.5–8.5 (optimum, 7.5). Grows at NaCl concentrations of 1.5–20 % (w/v). Positive for urease, arginine dihydrolase and nitrate reduction, and negative for hydrolysis of aesculin and gelatin, indole production, glucose acidification, L-phenylalanine deaminase and β-galactosidase. Utilizes glucose, *myo*-inositol, maltose, citrate, L-asparagine, L-aspartic acid, L-glutamic acid, glycerol and L-proline as sole carbon sources, but not adonitol, amylum, L-arabinose, D-cellulose, α-cyclodextrin, β-cyclodextrin, dextrin, L-fructose, D-galactose, D-lactose, D-mannose, melibiose, raffinose, L-rhamnose, D-ribose, sucrose, trehalose, ethanol, D-mannitol, methanol, acetate, glycine, L-histidine, L-ornithine, L-phenylalanine or D-serine. With the API ZYM system, positive for alkaline phosphatase, esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase and naphthol-AS-BI-phosphohydrolase, weakly positive for esterase (C4) and α-chymotrypsin, and negative for acid phosphatase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Sensitive to (μg per disc, unless indicated otherwise): carbenicillin (100), chloramphenicol (30) and penicillin (10 U), but resistant to amikacin (30), ampicillin (10), erythromycin (15), gentamicin (10), nalidixic acid (30), norfloxacin (10) and streptomycin (10). Polar lipids consist of diphosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylcholine, phosphatidylinositol and three unknown phospholipids. Major fatty acids are $C_{18:1}\omega7c$, $C_{18:1}$ 2-OH and $C_{16:0}$. The isoprenoid quinone is Q-10. The DNA G+C content of the type strain is 62.3 mol%.

The type strain, YIM D812^T (=CCTCC AA 208037^T=DSM 21160^T), was isolated from a salt mine of Fenggang in Yunnan, south-west China.

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