Mangrovimonas yunxiaonensis gen. nov., sp. nov., isolated from mangrove sediment

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A Gram-negative, short-rod-shaped, orange-pigmented bacterium, strain LYYY01^T, was isolated from a mangrove sediment sample collected from Yunxiao mangrove National Nature Reserve, Fujian Province, China. 16S rRNA gene sequence comparisons showed that strain LYYY01^T is a member of the family *Flavobacteriaceae*, forming a distinct lineage with species of the genera *Meridianimaribacter*, *Sediminibacter*, *Gelidibacter* and *Subsaximicrobium*. The 16S rRNA gene sequence similarity between strain LYYY01^T and the type strains of related species ranged from 93.9 to 90.9 %. Growth was observed at temperatures from 10 to 38 °C, at salinities from 1 to 7 % and at pH from 6 to 10. The DNA G+C content of the strain was 38.6 mol% and the major respiratory quinone was menaquinone-6 (MK-6). The major fatty acids were iso-$C_{15:1}$ (27.6 %), iso-$C_{15:0}$ (24.0 %), iso-$C_{17:0}$ 3-OH (12.0 %) and iso-$C_{16:0}$ 3-OH (6.2 %). According to its morphology, physiology, fatty acid composition and 16S rRNA gene sequence data, strain LYYY01^T is considered to represent a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Mangrovimonas yunxiaonensis* gen. nov., sp. nov. is proposed. The type strain of *Mangrovimonas yunxiaonensis* is LYYY01^T (=CGMCC 1.12280^T=LMG 27142^T).

In an attempt to investigate the algicidal bacteria in the sediment of Zhangjiang estuary mangrove, Fujian Province, China, many bacterial strains were isolated and characterized taxonomically. This study focused on one of these isolates, designated strain LYYY01^T, with algicidal activity against the harmful algal species *Alexandrium tamarense*. Comparative 16S rRNA gene sequence analysis indicated that strain LYYY01^T formed a clade with the genus *Meridianimaribacter* (Wang *et al.*, 2010) within the family *Flavobacteriaceae*. The family *Flavobacteriaceae*, proposed by Jooste (1985), contains, at the time of writing, 101 validly named genera (http://www.bacterio.cict.fr/). Accordingly, the aim of the present work was to determine the exact taxonomic position of strain LYYY01^T by using polyphasic characterization, including determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Strain LYYY01^T was isolated from a surface sediment sample collected in November 2011 at a depth of 20 cm from Yunxiao mangrove National Nature Reserve (23° 55′ N 117° 24′ E), Fujian Province, China. About 2.0 g of sediment slurry was added to fresh marine broth 2216 (MB; Difco), enrichment was conducted in a rotary shaker (150 r.p.m.) at 28 °C for 2 weeks and the culture was kept in the dark. The enrichment culture was diluted using sterile seawater and spread onto marine agar 2216 (MA; Difco). Strain LYYY01^T was purified on fresh MA three times and stored at −80 °C in MB supplemented with 10 % (v/v) glycerol.

Genomic DNA was extracted according to the method of Ausubel *et al.* (1995). The 16S rRNA gene sequence of strain LYYY01^T was amplified by PCR using primers P27F and P1492R (DeLong, 1992). Purification of the PCR product was carried out according to the protocol of the TIANquick midi purification kit (Tiangen). The PCR product was cloned into vector pMD19-T and sequenced. Sequences of related taxa were obtained from the GenBank and the EzTaxon databases. Phylogenetic analysis was performed using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignment of the data by DNAMAN (version 5.1). Evolutionary distances and clustering were determined by using the neighbour-joining (Saitou & Nei, 1987) method,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LYYY01^T is JQ937283.

and were evaluated by using bootstrap values based on 1000 replications.

The nearly full-length 16S rRNA gene sequence (1448 bp) of strain LYYY01^T was determined. Phylogenetic analysis of strain LYYY01^T indicated that it belonged to the family *Flavobacteriaceae* (Fig. 1). In the phylogenetic tree based on the neighbour-joining algorithm, strain LYYY01^T joined the phylogenetic clade comprising *Meridianimaribacter flavus* NH57N^T, with which it exhibited highest 16S rRNA gene sequence similarity (93.9 %). Lower similarities were shown to other members of the family *Flavobacteriaceae* (<93.5 %). Levels of sequence similarity among the closest 50 strains were further determined using the Eztaxon-e server (Kim *et al.*, 2012). These relatively low levels of 16S rRNA gene sequence similarity to its most closely related genera suggested that strain LYYY01^T may represent a novel species of a new genus.

Cell morphology and motility were observed by using transmission electron microscopy (model JEM-2100HC; JEOL; Fig. 2) and phase-contrast light microscopy (model 50i; Nikon), with cells from the early exponential phase grown on MA. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. The presence of flexirubin-type pigments was assessed using the bathochromic shift test with 20 % (w/v) KOH, as described by Bernardet *et al.* (2002). Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Anaerobic growth was checked on MA; the medium was autoclaved and cooled to room temperature under an anoxic atmosphere of N_2 (99.999 % purity). Culturing was conducted in triplicate in 50 ml anaerobic serum bottles sealed with thick butyl rubber stoppers and aluminium caps, and the cultures were incubated statically in the dark at 28 °C for 21 days. Growth was tested at 0–45 °C (at 0 °C, 4 °C, 10–25 °C at 5 °C intervals, 28 °C, and 37–45 °C at 1 °C intervals) in MB. Growth was tested at pH 3.0–12.0 (at 1 pH unit intervals), adjusted as described by Lai *et al.* (2010). Verification of the pH after autoclaving revealed only minor changes. The NaCl concentration range and optimum for growth were determined in NaCl-free 2216 medium (per litre distilled water: 5.0 g tryptone and 1.0 g yeast extract, pH 7.6–7.8), supplemented with various concentrations (w/v) of NaCl (0–5.0 % at 0.5 % intervals and 5.0–12.0 % at 1 % intervals). Catalase activity was determined by addition of 3 % hydrogen peroxide to exponential-phase colonies, and the oxidase reaction was tested by using oxidase reagent (bioMérieux). Hydrolysis of starch, Tweens 20, 40 and 80, and casein was tested using MA, at 0.5 % (w/v) for starch and at 1 % (w/v) for the

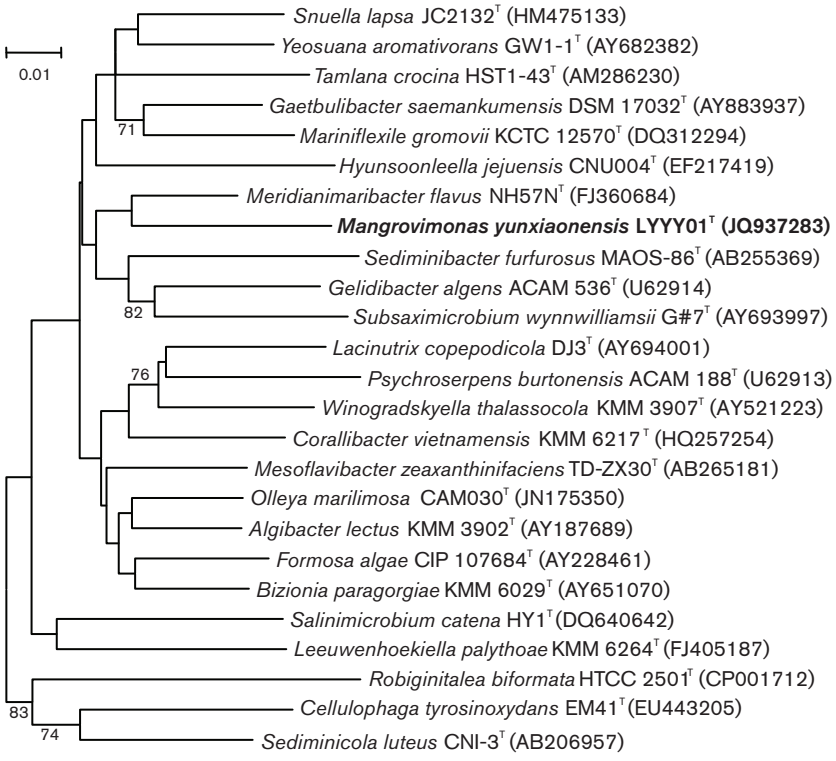

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain LYYY01^T among representative members of related genera in the family *Flavobacteriaceae*. Bootstrap values above 70 % (expressed as percentages of 1000 replications) are given at nodes. Bar, 0.01 nt substitution rate (K_{nuc}) units.

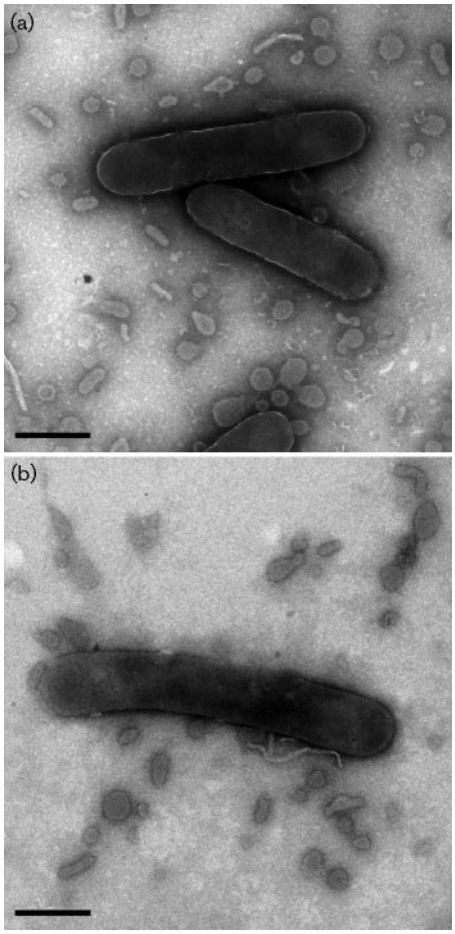

Fig. 2. Transmission electron micrographs of cells of strain LYYY01^T grown on marine agar 2216 medium for 24 h at 28 °C. Bars, 0.5 μm.

other substrates. DNA was tested according to the protocol of Cowan & Steel (1993). Results were examined twice after growth on agar plates for 3 and 5 days. Other biochemical tests were carried out using API 20NE, API 20E and API ZYM strips (bioMérieux) according to the manufacturer's instructions, except adjusting the NaCl concentration in all tests to 3.0 %. All above-mentioned tests were evaluated at 28 °C. Susceptibility to antibiotics was tested on MA at 28 °C for 2 days by using the following discs (Oxoid): ampicillin (10 μg), carbenicillin (100 μg), ceftriaxone (30 μg), cephradine (30 μg), cefoperazone (75 μg), chloramphenicol (30 μg), cephalexin (30 μg), cephazolin (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), doxycycline hydrochloride (30 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (2 μg), metronidazole (5 μg), minocycline (30 μg), norfloxacin (10 μg), ofloxacin (5 μg), oxacillin (1 μg), penicillin G (10 μg), piperacillin (100 μg), polymyxin B (300 IU), rifampicin (5 μg), streptomycin (10 μg), tetracycline (30 μg), trimethoprim (25 μg) and vancomycin (30 μg). The physiological and biochemical characteristics of strain LYYY01^T are given in the genus and species descriptions and in Table 1.

To determine the DNA G+C content, genomic DNA was extracted from cells cultured on MA for 3 days at 28 °C and analysed by reversed-phase HPLC (Tamaoka & Komagata, 1984). The DNA G+C content of strain LYYY01^T was 38.6 mol%.

For cellular fatty acid analysis, fatty acids in cells of strain LYYY01^T and *M. flavus* NH57N^T grown on MA plates at 28 °C for 48 h were extracted, saponified and esterified at the same time according to the standard protocol of Sasser (1990). The fatty acids were analysed using a gas chromatograph (6850; Agilent), and peaks were identified with the MIDI software (version 6.0). As shown in Table 2, the major fatty acids of strain LYYY01^T were iso-$C_{15:1}$ (27.6 %), iso-$C_{15:0}$ (24.0 %), iso-$C_{17:0}$ 3-OH (12.0 %) and iso-$C_{16:0}$ 3-OH (6.2 %), which accounted for 69.8 % of the total. Compared with *M. flavus* NH57N^T, strain LYYY01^T possessed iso-$C_{12:0}$ and $C_{12:1}$ at 11–12, but did not possess iso-$C_{11:0}$ or iso-$C_{11:0}$ 3-OH. The content of summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) in strain LYYY01^T (2.7 %) was lower than that in strain NH57N^T (8.1 %). Members of the family *Flavobacteriaceae* typically have high levels of branched-chain and 3-hydroxy C_{15}–C_{17} fatty acids (Bowman *et al.*, 1998); strain LYYY01^T possesses large amounts of iso-$C_{17:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, iso-$C_{15:0}$ 3-OH and branched-chain fatty acids, suggesting that it is a member of the family *Flavobacteriaceae*.

Analysis of the respiratory quinone was carried out by the identification service of the DSMZ. The major respiratory quinone of strain LYYY01^T was menaquinone-6 (MK-6), in accordance with all members of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). This also indicated that strain LYYY01^T is a member of the family *Flavobacteriaceae*.

Comparisons of strains LYYY01^T and *M. flavus* NH57N^T revealed many phenotypic differences. These included differences in the degradation of Tweens 20, 40 and 80 as well as acid production from glucose, mannose, inosine, sorbitol and arabinose. Strain LYYY01^T has a flexirubin-type pigment, which is absent in *M. flavus* NH57N^T. Compared with *M. flavus* NH57N^T, strain LYYY01^T exhibited wider pH and salinity ranges for growth. Strain LYYY01^T was susceptible to cephazolin and co-trimoxazole while *M. flavus* NH57N^T was resistant. In addition, strain LYYY01^T could be distinguished from its closest relatives based on various phenotypic differences. The requirement of Na^+ ions for growth distinguished strain LYYY01^T from members of the genus *Gelidibacter*. Weak acid production from mannose, inosine and sorbitol and inability to hydrolyse casein or DNA differentiated the novel strain from the other strains. The dominant fatty acids of strain LYYY01^T and *Sediminibacter*, *Gelidibacter* and *Subsaximicrobium* species showed huge differences.

Table 1. Differential phenotypic characteristics of strain LYYY01^T and closely related members of the family *Flavobacteriaceae*

Taxa: 1, strain LYYY01^T (*Mangrovimonas yunxiaonensis* gen. nov., sp. nov.); 2, *Meridianimaribacter flavus* NH57N^T (data from this study, except where indicated); 3, *Sediminibacter* (data from Khan *et al.*, 2007); 4, *Gelidibacter* (*n*=4; Kwon *et al.*, 2006; Bowman & Nichols, 2005); 5, *Subsaximicrobium* (*n*=2; Bowman & Nichols, 2005; Kwon *et al.*, 2006). +, Positive; −, negative; W, weakly positive; ND, no data available; V, variable.

Characteristic	1	2	3	4	5
Gliding motility	+	+	−	+	+
Colour of cell mass	Orange	Yellow	Brown	Orange-yellow	Yellow
Flexirubin-type pigments	+	−	−	−	−
Requirement for Na^+	+	+	+	−	+
Growth temperature (°C)	10–38	9–37*	10–37	0–37	−2 to 25
Optimum temperature (°C)	28–37	30–37*	25–30	15–18	1–20
Salinity range (%, w/v)	1–7	0.5–4.0*	1–6	0–15	0.5–12
Optimal salinity (%)	2–5	0.5–2.0*	3–4	ND	ND
pH	6.0–10.0	6.5–8.5*	ND	ND	ND
Optimal pH	7.0–9.0	7.8*	ND	ND	ND
API 20E results					
β-Galactosidase	−	+	W	V	−
Urease activity	+	−	−	−	−
Acid production from:					
Glucose	−	W	−	+	−
Mannose	W	−	+	V	V
Inosine	W	−	+	ND	ND
Sorbitol	W	−	+	−	ND
Arabinose	−	W	ND	+	+
API 20NE results					
β-Glucosidase	W	+	W	V	V
API ZYM results					
Esterase lipase (C8)	W	+	ND	ND	ND
Chymotrypsin	−	+	ND	ND	ND
Susceptibility to:					
Cephazolin	+	−	ND	ND	ND
Co-trimoxazole	+	−	ND	ND	ND
Degradation of:					
Casein	−	−	+	V	+
DNA	−	−	+	−	V
Gelatin	+	+	+	V	+
Starch	W	W	+	V	+
Tween 20	+	−	ND	ND	ND
Tween 40	+	W	ND	ND	ND
Tween 80	W	−	ND	V	+
DNA G+C content (mol%)	38.6	32.7	39	36–42	38–41

*Data from Wang *et al.* (2010).

Overall, on the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons (Tables 1 and 2 and Fig. 1), strain LYYY01^T should be placed in a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Mangrovimonas yunxiaonensis* gen. nov., sp. nov. is proposed.

Description of *Mangrovimonas* gen. nov.

Mangrovimonas [Man.gro.vi.mo′nas. N.L. n. *mangrovum* mangrove; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Mangrovimonas* a unit (bacterium) isolated from a mangrove].

Cells are Gram-negative, aerobic, short-rod-shaped and motile by gliding. Orange colonies are formed on MA plates. Flexirubin pigments are formed. Catalase- and oxidase-positive. The major respiratory quinone is MK-6. The main fatty acids are iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{16:0}$ 3-OH, iso-$C_{15:0}$ 3-OH and iso-$C_{16:0}$. As determined by 16S rRNA gene sequence analysis, the genus is a member of the family *Flavobacteriaceae*. The type species is *Mangrovimonas yunxiaonensis*.

Table 2. Cellular fatty acid contents of strain LYYY01^T and closely related members of the family *Flavobacteriaceae*

Taxa: 1, strain LYYY01^T (*Mangrovimonas yunxiaonensis* gen. nov., sp. nov.); 2, *Meridianimaribacter flavus* NH57N^T (data from this study); 3, *Sediminibacter* (data from Khan *et al.*, 2007); 4, *Gelidibacter* (*n*=4; Bowman & Nichols, 2005); 5, *Subsaximicrobium* (*n*=2; Bowman & Nichols, 2005). For taxa 4 and 5, values shown are the range of percentages for all species in the genus or for all strains of the species. tr, Trace (<0.5 %); ND, not detected.

Fatty acid	1	2	3	4	5
iso-$C_{13:0}$	0.7	ND	ND	ND	ND
iso-$C_{13:0}$ 3-OH	tr	tr	ND	ND	ND
$C_{13:1}$ at 12–13	tr	tr	ND	ND	ND
$C_{14:0}$	0.6	0.5	ND	tr to 0.6	tr to 0.5
iso-$C_{14:0}$	2.3	1.4	tr	tr to 2.8	tr to 0.7
iso-$C_{14:0}$ 3-OH	0.7	0.5	ND	ND	ND
br-$C_{14:1}$	ND	ND	ND	tr to 1.7	tr to 0.5
anteiso-$C_{15:0}$	0.6	0.6	2.0	10.5–17.7	9.9–17.1
$C_{15:0}$ 2-OH	tr	tr	tr	ND	ND
$C_{15:0}$ 3-OH	1.3	1.0	ND	tr to 1.5	0–0.8
iso-$C_{15:0}$	24.0	21.1	39.0	3.4–8.8	7.4–10.4
iso-$C_{15:0}$ 3-OH	4.8	5.6	6.0	2.2–6.2	2.4–5.4
anteiso-$C_{15:1}$	tr	tr	ND	11.8–16.6	5.9–14.7
iso-$C_{15:1}$	27.6	25.6	25.0	5.3–11.4	6.7–13.4
$C_{16:0}$	2.2	2.3	2.0	1.2–2.2	1.9–2.7
$C_{16:0}$ 3-OH	0.7	0.8	ND	ND	ND
iso-$C_{16:0}$	4.4	2.2	tr	1.4–4.4	ND
iso-$C_{16:0}$ 3-OH	6.2	7.9	1.0	4.1–12.2	8.8–13.7
iso-$C_{16:1}$	2.2	0.7	tr	ND	ND
$C_{17:0}$	tr	tr	ND	ND	ND
anteiso-$C_{17:0}$	ND	tr	ND	ND	ND
$C_{17:0}$ 3-OH	0.8	tr	ND	ND	ND
iso-$C_{17:0}$	ND	tr	ND	ND	ND
iso-$C_{17:0}$ 3-OH	12.0	13.1	9.0	0–3.1	1.0–3.4
$C_{17:1}\omega6c$	tr	tr	tr	ND	ND
$C_{18:0}$	1.2	2.0	ND	0.5–1.2	1.2–2.1
$C_{18:1}\omega9c$	1.0	1.0	ND	ND	ND
Summed feature*					
3	2.7	8.1	9.0	4.3–9.5	9.5–13.4
8	2.0	2.5	ND	ND	ND
9	tr	ND	ND	ND	ND

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3, $C_{16:1}\omega6c/\omega7c$; summed feature 8, $C_{18:1}\omega7c/\omega6c$; summed feature 9, iso-$C_{17:1}\omega9c$ and/or $C_{16:0}$ 10-methyl.

Description of *Mangrovimonas yunxiaonensis* sp. nov.

Mangrovimonas yunxiaonensis (yun.xi.a.o.nen′sis. N.L. fem. adj. *yunxiaonensis* of or belonging to Yunxiao mangrove National Nature Reserve, Fujian Province, China).

Exhibits the following properties in addition to those described for the genus. Cells are 0.9–2.5 μm in length and 0.3–0.5 μm in diameter. Growth occurs at 10–38 °C, with an optimum at 28–37 °C. Growth occurs at NaCl concentrations of 1–7 % (w/v), with optimal growth at 2–5 %. Growth does not occur in the absence of NaCl. Growth occurs at pH 6–10, with optimal growth at pH 7–9. Colonies on MA are smooth, moist, circular, shiny with entire edges and 1–2 mm in diameter after 3 days incubation at 28 °C. Possesses a flexirubin-type pigment that exhibits an immediate colour shift from orange to red. Positive for hydrolysis of gelatin, Tweens 20 and 40, and production of acetoin. Weakly positive for hydrolysis of starch and Tween 80. Negative for hydrolysis of DNA and casein, reduction of nitrate, and production of H_2S and indole. Positive for urease activity, weakly positive for β-glucosidase (aesculinase hydrolysis), tryptophan deaminase, and utilization of inositol, sorbitol, sucrose and mannose in API 20NE and API 20E strips. Negative for β-galactosidase, arginine dihydrolase, ornithine decarboxylase and lysine decarboxylase activities and utilization of glucose, mannitol, rhamnose, melibiose, amygdalin, arabinose, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid (API 20NE and API 20E data). Positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, weakly positive for esterase (C4), esterase lipase (C8), cystine arylamidase and trypsin activities, but negative for α-chymotrypsin, lipase (C14), α-galactosidase, α-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities in API ZYM strips. Susceptible to co-trimoxazole, ofloxacin, carbenicillin, cephradine, doxycycline hydrochloride, ampicillin, chloramphenicol, ciprofloxacin, cefoperazone, erythromycin, clindamycin, ceftriaxone, cephalexin, lincomycin, minocycline, norfloxacin, tetracycline, piperacillin, penicillin G, rifampicin, cephazolin and vancomycin, but resistant to kanamycin, polymyxin B, metronidazole, streptomycin, oxacillin and gentamicin. The predominant fatty acids (>5 % of the total) are iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and iso-$C_{16:0}$ 3-OH. The major respiratory quinone is menaquinone-6. Other phenotypic characteristics are given in Table 1.

The type strain, LYYY01^T (=CGMCC 1.12280^T=LMG 27142^T), was isolated from a mangrove sediment sample collected from Yunxiao mangrove National Nature Reserve, Fujian Province, China. The DNA G+C content of the type strain is 38.6 mol%.

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