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

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A Gram-negative, short-rod-shaped, orange-pigmented bacterium, strain LYYY01T, 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 LYYY01T 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 LYYY01T 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-C15 : 1(27.6 %), iso-C15 : 0(24.0 %), iso-C17 : 0 3-OH (12.0 %) and iso-C16 : 0 3-OH (6.2 %). According to its morphology, physiology, fatty acid composition and 16S rRNA gene sequence data, strain LYYY01T 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 LYYY01T (=CGMCC 1.12280T=LMG 27142T).

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 LYYY01T, with algicidal activity against the harmful algal species Alexandrium tamarense. Comparative 16S rRNA gene sequence analysis indicated that strain LYYY01T 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 LYYY01T by using polyphasic characterization, including determination of phenotypic properties and a detailed phylogenetic analysis based on 16S rRNA gene sequences.

Strain LYYY01T 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 LYYY01T 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 LYYY01T 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.
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. 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 for 3 days. Colony morphology, size and colour were examined from cultures grown on MA. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days. Colony morphology, size and colour were examined from cultures grown on MA for 3 days.

**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 ($K_{sub}$) units.
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), cephadine (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), pipercillin (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 LYYY01T 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 LYYY01T was 38.6 mol%.

For cellular fatty acid analysis, fatty acids in cells of strain LYYY01T and M. flavus NH57NT 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 LYYY01T were iso-C₁₅ : 1 (27.6%), iso-C₁₅ : 0 (24.0%), iso-C₁₇ : 0 3-OH (12.0%) and iso-C₁₆ : 0 3-OH (6.2%), which accounted for 69.8% of the total. Compared with M. flavus NH57NT, strain LYYY01T possessed iso-C₁₂ : 0 and C₁₂ : 1 at 11–12, but did not possess iso-C₁₁ : 0 or iso-C₁₁ : 0 3-OH. The content of summed feature 3 (comprising C₁₆ : 1ω6c and/or C₁₆ : 1ω7c) in strain LYYY01T (2.7%) was lower than that in strain NH57NT (8.1%). Members of the family Flavobacteriaceae typically have high levels of branched-chain and 3-hydroxy C₁₅–C₁₇ fatty acids (Bowman et al., 1998); strain LYYY01T possesses large amounts of iso-C₁₇ : 0 3-OH, iso-C₁₆ : 0 3-OH, iso-C₁₅ : 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 LYYY01T was menaquinone-6 (MK-6), in accordance with all members of the family Flavobacteriaceae (Bernardet et al., 2002). This also indicated that strain LYYY01T is a member of the family Flavobacteriaceae.

Comparisons of strains LYYY01T and M. flavus NH57NT 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 LYYY01T has a flexirubin-type pigment, which is absent in M. flavus NH57NT. Compared with M. flavus NH57NT, strain LYYY01T exhibited wider pH and salinity ranges for growth. Strain LYYY01T was susceptible to cephalin and cotrimoxazole while M. flavus NH57NT was resistant. In addition, strain LYYY01T could be distinguished from its closest relatives based on various phenotypic differences. The requirement of Na⁺ ions for growth distinguished strain LYYY01T 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 LYYY01T and Sediminibacter, Gelidibacter and Subsaximicrobium species showed huge differences.

**Fig. 2.** Transmission electron micrographs of cells of strain LYYY01T grown on marine agar 2216 medium for 24 h at 28 °C. Bars, 0.5 μm.
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 LYYY01T 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-C15 : 1, iso-C15 : 0, iso-C17 : 03-OH, iso-C16 : 03-OH, iso-C15 : 0 3-OH and iso-C16 : 0. As determined by 16S rRNA gene sequence analysis, the genus is a member of the family Flavobacteriaceae. The type species is Mangrovimonas yunxiaonensis.
Mangrovimonas yunxiaonensis gen. nov., sp. nov.

**Description of Mangrovimonas yunxiaonensis sp. nov.**


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 H₂S and indole. Positive for urease activity, weakly positive for β-glucosidase (ascellulase 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 β-chymotrypsin, lipase (C14), β-galactosidase, 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, cephalaxin, lincomycin, minocycline, norfloxacin, tetracycline, piperacillin, penicillin G, rifampicin, cephalazin and vancomycin, but resistant to kanamycin, polymyxin B, metronidazole, streptomycin, oxacillin and gentamicin. The predominant fatty acids (>5 % of the total) are iso-C₁₅:0 3-OH, iso-C₁₅:0 3-OH and/or C₁₆:0 10-methyl.

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References


