**Fluviicola hefeinensis** sp. nov., isolated from the wastewater of a chemical factory

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A Gram-negative, strictly aerobic, yellow–orange-pigmented, motile, short rod-shaped, catalase-positive, oxidase-negative bacterium, strain MYL-8T, was isolated from wastewater of the Jin Tai Chemical Factory in Hefei, China. Strain MYL-8T grew optimally at 30 °C, in the absence of NaCl and at pH 7. Menaquinone 6 (MK-6) was the sole respiratory quinone and the major fatty acids were iso-C15:0, iso-C15:1G, iso-C17:0 3-OH and summed feature 3 (C16:1 \± 7c and/or iso-C15:0 2-OH). The polar lipid profile was composed predominantly of unidentified polar lipids and aminolipids. Minor amounts of phosphatidylethanolamine and unidentified phospholipids were also detectable. The DNA G+C content of strain MYL-8T was 43.5 mol%. The 16S rRNA gene sequence of strain MYL-8T showed the highest similarity to that of *Fluviicola taffensis* RW 262T (97.03%), followed by *Wandonia halotis* Haldis-1-1T (92.05%), *Lishizhenia caseinilytica* UST040201-001T (91.43%) and *Lishizhenia tianjinensis* JCM 15141T (90.61%). DNA–DNA relatedness between strain MYL-8T and *F. taffensis* RW 262T was 21.35 ± 0.90%. On the basis of phenotypic, chemotaxonomic, genomic and phylogenetic data, strain MYL-8T is considered to represent a novel species of the genus *Fluviicola*, for which the name *Fluviicola hefeinensis* sp. nov. is proposed. The type strain is MYL-8T (=KACC 16597T=CCTCC AB 2013168T).

The genus *Fluviicola*, grouped in the family *Cryomorphaceae* in the class *Flavobacteria*, was proposed by O’Sullivan et al. (2005). At the time of writing, the family *Cryomorphaceae* includes nine genera: *Brumimicrobium* (Bowman et al., 2003), *Cryomorpha* (Bowman et al., 2003), *Crocinitomix* (Bowman et al., 2003), *Owenweeksia* (Lau et al., 2005), *Fluviicola* (O’Sullivan et al., 2005), *Lishizhenia* (Lau et al., 2006), *Wandonia* (Lee et al., 2010), *Salinirepens* (Muramatsu et al., 2012) and *Phaeocystidibacter* (Zhou et al., 2013), most of which are of marine origin. Members of the genus *Fluviicola* are characterized as strictly aerobic, Gram-negative, yellow–orange-pigmented, motile, rod-shaped, catalase-positive and oxidase-negative bacteria. At the time of writing, the genus *Fluviicola* includes only one recognized species, *Fluviicola taffensis* (O’Sullivan et al., 2005). From wastewater of the Jin Tai Chemical Factory in Hefei, China, a yellow–orange-pigmented bacterial strain, designated MYL-8T, was isolated. The strain was subjected to a polyphasic taxonomic investigation, including determination of chemotaxonomic and phenotypic properties and genomic relatedness and phylogenetic analysis. On the basis of the results obtained in this study, we propose that strain MYL-8T should be placed in the genus *Fluviicola* as the type strain of a novel species, *Fluviicola hefeinensis* sp. nov.

The wastewater collected for micro-organism isolation was diluted 10-fold with sterilized double-distilled water, spread on mineral salts medium (MSM) agar (1.5 g K2HPO4, 0.5 g KH2PO4, 0.2 g MgSO4.7H2O, 1.0 g NaCl, 1.0 g NH4NO3 and 15.0 g agar per litre water, pH 7.2; Wang et al., 2011) and incubated at 30 °C for 6 days. Colonies grown on the plates were picked and cultured in Luria–Bertani (LB)
medium (Berti, 1951) at 30 °C for 3–4 days. Strain MYL-8T was obtained after several streakings and transfers on LB plates. The pure culture of strain MYL-8T was preserved at −80 °C in LB medium with 15 % (v/v) glycerol.

Gram staining was performed following the method described by Beveridge et al. (2007). Cell morphology was determined by transmission electron microscopy (model H-7650; Hitachi). Motility was established by phase-contrast microscopy of colonies after cultivation on LB agar at 30 °C for 2 days. All physiological and biochemical tests on strain MYL-8T were performed simultaneously with the reference strain F. taffensis RW 262T which was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. (DSMZ). F. taffensis RW 262T was cultured on CYT agar (1 g pancreatic digest of casein, 0.5 g CaCl2, 2H2O, 0.5 g MgSO4·7H2O, 0.5 g yeast extract and 15 g agar per litre water, pH 7.2; Holmes, 1992), while strain MYL-8T was cultured on either CYT agar or LB agar. Catalase activity was determined by assessing bubble production in 3.0 % (w/v) H2O2 (Zhang et al., 2012). Oxidase activity was tested using oxidase reagent (bioMérieux) according to the instructions of the manufacturer. The presence of flexirubin-type pigments was detected by using 20 % KOH (Fautz & Reichenbach, 1980). The temperature for growth was determined on LB agar at 4, 10, 15, 20, 25, 30, 37 and 45 °C. Tolerance of NaCl was tested in modified LB medium containing 0, 1, 2.5, 5 and 10 % (w/v) NaCl. Antibiotic susceptibility was examined by placing different antibiotic discs on LB agar. The tested discs (Oxoid) contained: ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), novobiocin (5 μg), rifampicin (5 μg), penicillin G (1 μg), streptomycin (10 μg) and tetracycline (30 μg). Additional biochemical tests were performed using API 20NE and API ZYM kits (bioMérieux) according to the manufacturer’s instructions.

Analysis of respiratory quinones by HPLC and polar lipids by two-dimensional TLC were carried out by the identification service of the DSMZ (Braunschweig, Germany). Respiratory quinones and polar lipids were extracted and analyzed as described by Tindall (1990a, b) and Tindall (2007), respectively. Cellular fatty acids analysis of strain MYL-8T and strain RW262T was performed by using the Sherlock MIS (MIDI Inc, Newark, USA) system, which was carried out by the identification service of the DSMZ (Braunschweig, Germany) as described by Miller (1982) and Kuykendall et al. (1988). Both strains were cultivated on CYT agar at 20 °C for 3 days.

The DNA G + C content was determined by reversed-phase HPLC according to Mesbah et al. (1989). Genomic DNA from strain MYL-8T was extracted and purified according to standard procedures (Tel-Zur et al., 1999). PCR primer pair 27F/1492r was used to amplify the consens 16S rRNA gene (Lane, 1991; Marchesi et al., 1998). The amplified PCR products were cloned into the pMD18-T vector (Takara), transformed into competent Escherichia coli DH5α cells and then sequenced using an automated sequencer (Applied Biosystems model 3730). The 16S rRNA gene sequence was compared to known sequences found in GenBank using the BLAST program of the National Center for Biotechnological Information (http://www.ncbi.nlm.nih.gov/BLAST/) and also identified in EzTaxon server 2.1 (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Alignment with 16S rRNA gene sequences from GenBank was performed using CLUSTAL_X (Thompson et al., 1997) with default settings. Phylogenesis was analysed with MEGA version 4.0 software (Tamura et al., 2007) and distances were calculated using Kimura’s two-parameter distance model. Phylogenetic trees were reconstructed using the neighbour-joining, minimum-evolution and maximum-parsimony methods. DNA–DNA hybridization between strain MYL-8T and F. taffensis RW 262T was carried out using the method described by Ezaki et al. (1989).

Strain MLY-8T was Gram-negative, strictly aerobic, yellow–orange-pigmented and motile by gliding. Cells were rod-shaped (0.3–0.5 μm in diameter and 1.1–1.8 μm long) when cultured on LB agar at 30 °C for 2 days (Fig. S1, available in IJSEM Online). Strain MLY-8T grew well on LB and CYT agar, but not on marine agar 2216 (MA). Colonies of strain MLY-8T on LB agar were 1–3 mm in diameter, circular, flat and transparent, with a yellow–orange colour, after 4 days of incubation at 30 °C. Strain MLY-8T grew at 4–37 °C, but not at 45 °C. The optimal temperature was 30 °C. Growth occurred at pH 5.0–10.0, with an optimum at pH 7.0. The range of NaCl concentration for growth of the strain was 0–2.5 %, with optimum growth in the absence of NaCl. Using the API ZYM and API 20NE kits, it was found that strain MLY-8T could not utilize glucose, arabinose, mannose, mannotol, N-acetylglucosamine, maltose, gluconate, caprate, adipate, malate, citrate or phenylacetate. Starch, gelatin, DNA, arginine and aesculin were not hydrolysed. Strain MLY-8T was positive for activities of catalase, alkaline phosphatase, C4 esterase, C8 esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, while it was negative for activities of oxidase, β-galactosidase, xylanase, urease, C14 lipase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, x-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and β-fucosidase. It was weakly positive for reduction of nitrate and nitrite. Differential phenotypic and biomedical characteristics between strain MYL-8T and closely related type strains are summarized in Table 1.

The major fatty acid profile of strain MYL-8T was similar to that of F. taffensis RW 262T analysed in parallel under the same cultivation conditions in the present study (Table S1). The major fatty acids of strain M-8T were iso-C15 : 0 (39.96 %), iso-C15 : 1 G (18.14 %), iso-C17 : 0 3-OH (13.67 %) and summed feature 3 (C16 : 1 v c 7c and/or iso-C15 : 0 2-OH; 11.17 %). The presence of the major fatty acid profiles were found between strain MYL-8T and the only member of the genus Fluvicola (O’Sullivan et al., 2005). However, differences in fatty acid profiles were found between strain MYL-8T and F. taffensis RW 262T (Table S1). The major polar lipids

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of strain MLY-8T were unidentified lipids (L4 and L6) and aminolipids (AL1 and AL2). Minor amounts of phosphatidylethanolamine and unidentified phospholipids were also detectable (Fig. S2). However, phospholipids were not detected in F. taffensis RW 262T. The sole respiratory quinone of strain MLY-8T was menaquinone 6 (MK-6), which is consistent with the other members of the family Cryomorphaceae, and was negative for oxidase. These phenotypic and biochemical properties were in agreement with those of the genus Fluviicola, but different from those of the genera Wandonia and Lishizhenia. Strain MLY-8T was distinguished from F. taffensis RW 262T, the only known member of the genus Fluviicola, by several phenotypic, biochemical and chemotaxonomic characteristics (Table 1). For example, strain MLY-8T grew well on LB agar and in the presence of 1 % NaCl and could grow on CYT and in the absence of NaCl, could not grow on MA and was negative for oxidase. These phenotypic and biochemical properties were in agreement with those of the genus Fluviicola, but different from those of the genera Wandonia and Lishizhenia. Strain MLY-8T was distinguished from F. taffensis RW 262T, the only known member of the genus Fluviicola, by several phenotypic, biochemical and chemotaxonomic characteristics (Table 1). For example, strain MLY-8T grew well on LB agar and in the presence of 1 % NaCl and could grow at 37 °C, while F. taffensis RW 262T could not. Moreover, F. taffensis RW 262T could degrade gelatin weakly, while strain MLY-8T could not. Therefore, on the basis of the polyphasic taxonomic approach described here, we consider strain MLY-8T to merit classification within a novel species of the genus Fluviicola, for which we propose the name Fluviicola hefeinensis sp. nov.

**Description of Fluviicola hefeinensis sp. nov.**

*Fluviicola hefeinensis* (he.fei.nen’sis. N.L. masc. adj. hefei-ensis pertaining to Hefei, from where the type strain was isolated).
Cells are aerobic, Gram-negative and motile by gliding. Flexirubin-type pigments are present. Cells are rod-shaped (0.3–0.5 μm in diameter and 1.1–1.8 μm long) when cultured on LB agar at 30 °C for 2 days. After 4 days of cultivation on LB agar at 30 °C, colonies are 1–3 mm in diameter, circular, flat and transparent, with a yellow–orange colour. Growth occurs at 4–37 °C (optimum at 30 °C), at pH 5.0–10.0 (optimum at 7.0) and with 0–2.5 % NaCl (optimum without NaCl). Growth occurs on LB agar and CYT agar, but not on MA. Positive for catalase, alkaline phosphatase, C4 esterase, C8 esterase lipase, C14 lipase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase N-acetyl-β-glucosaminidase, α-mannosidase and β-fucosidase. Weakly positive for reduction of nitrate and nitrite. Negative for hydrolysis of starch, gelatin, DNA, arginine and aesculin. Glucose acidification and indole liberation by lysogenic bacteria. The type strain is MYL-8T (=KACC 16597T=CCTCC AB 2013168T), isolated from wastewater of the Jin Tai Chemical Factory in Hefei, China. The DNA G+C content of the type strain is 43.5 mol%.

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