Flavobacterium dongtanense sp. nov., isolated from the rhizosphere of a wetland reed

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Two strains of Gram-reaction-negative, rod-shaped, non-spore-forming, non-motile, aerobic bacteria, designated LW30^T and LW29, were isolated from the rhizosphere of a wetland reed in Dongtan, Chongming Island, China. The strains formed pale-yellow colonies on R2A plates. Growth occurred at 4–37 °C (optimum 30 °C), at pH 6–9 (optimum pH 7–8) and in the presence of 0–3 % (w/v) NaCl (optimum 0–1 %). Oxidase and catalase activities and flexirubin-type pigments were absent. MK-6 was the major respiratory quinone. The major fatty acids were iso-C_{15:0}, C_{15:0}, iso-C_{15:1} G and iso-C_{17:1} ω9c. Strains LW30^T and LW29 could be differentiated from related species by several phenotypic characteristics. Phylogenetic analyses based on 16S rRNA gene sequences placed strains LW30^T and LW29 in the genus *Flavobacterium* with high sequence similarity to *Flavobacterium cheniae* NJ-26^T (94.0 %) and *Flavobacterium indicium* GPTSA 100-9^T (93.9 %). Together with *F. indicium* GPTSA 100-9^T, strains LW30^T and LW29 formed a distinct group in the phylogenetic tree. The DNA G+C content was 30 mol%. On the basis of the phylogenetic and phenotypic evidence, strains LW30^T and LW29 represent a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium dongtanense* sp. nov. is proposed. The type strain is LW30^T (=KCTC 22671^T =CCTCC AB 209201^T).

The genus *Flavobacterium* Bergey *et al.* (1923) belongs to the family *Flavobacteriaceae* in the phylum *Bacteroidetes* and its description was emended by Bernardet *et al.* (1996, 2002). Members of the genus *Flavobacterium* have been found in a wide range of habitats such as soil, sediment, fresh water, seawater and micromats and display a variety of physiological characteristics (Bernardet & Bowman, 2006). At the time of writing, the genus *Flavobacterium* comprises 86 species with validly published names.

Strains LW30^T and LW29 were isolated from the rhizosphere of a wetland reed in Dongtan, Chongming Island, China, by dilution-plating on R2A (Difco) agar plates. Cell morphology was examined by light microscopy (YS-2; Nikon) and transmission electron microscopy (H600; Hitachi) after negative staining with 1 % (w/v) phosphotungstic acid using cells grown on R2A agar for 2 days at 30 °C. Cell motility was determined by phase-contrast

microscopy (Eclipse TS 100; Nikon). The Gram reaction was determined using cells grown on R2A agar for 24 h at 30 °C according to the method described by Gerhardt *et al.* (1994). Growth in the presence of 0, 1, 2, 3 and 5 % (w/v) NaCl and at pH 5–10 (intervals of 1 pH unit) was tested in R2A broth and growth at 4, 10, 20, 30, 37 and 42 °C was tested on R2A agar for up to 1 week. The bathochromic-shift test, performed with 20 % (w/v) KOH (Fautz & Reichenbach, 1980), was used to detect flexirubin-type pigments. Catalase activity was observed by bubble production in a solution containing 3 % (v/v) hydrogen peroxide. Oxidase activity was determined by the oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck). Endospore formation and hydrolysis of starch and Tween 80 were determined as described by Dong & Cai (2001). Anaerobic growth was examined on R2A agar in an anaerobic test tube by using the AnaeroGen kit (Oxoid). Other physiological and biochemical properties were tested using API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions.

Strains LW30^T and LW29 had the same physiological, biochemical and culture characteristics; these are given in the species description and in Table 1. The two novel

Abbreviation: ECL, estimated chain length.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains LW30^T and LW29 are GU073293 and GU073292, respectively.

A supplementary table is available with the online version of this paper.

Table 1. Differential characteristics of strains LW30^T and LW29 and related members of the genus *Flavobacterium*

Taxa: 1, Strains LW30^T and LW29; 2, *F. indicum* GPTSA100-9^T (Saha & Chakrabarti, 2006); 3, *F. cheniae* NJ-26^T (Qu *et al.*, 2008); 4, *F. cucumis* R2A45-3^T (Weon *et al.*, 2007); 5, '*F. sasangense*' YC6274 (Yoon *et al.*, 2009). All strains are positive for hydrolysis of gelatin and are negative for flexirubin-type pigment, nitrate reduction and urease activity. +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5
Oxidase	–	+	+	+	+
Catalase	–	w	+	+	+
Tolerance of NaCl (% w/v)	0–3.0	0–2.0	0–0.8	0–2.0	0–1.0
Growth on:					
Nutrient agar	w	w	+	+	+
Trypticase soy agar	+	–	w	w	+
Gliding motility	–	–	+	+	+
Acid production from glucose	–	w	–	–	+
Growth at 37 °C	+	+	–	+	–
Hydrolysis of:					
Starch	–	+	w	+	+
Aesculin	–	ND	ND	+	–
Tween 80	+	–	–	ND	–
Utilization of:					
D-Glucose	–	w	–	–	ND
D-Mannitol	–	–	–	–	ND
API ZYM results					
Gluconate	–	ND	ND	–	ND
Esterase lipase (C8)	+	ND	–	+	w
Alkaline phosphatase	+	ND	+	+	+
Acid phosphatase	+	ND	ND	+	w
Valine arylamidase	+	ND	ND	+	+
Leucine arylamidase	–	ND	ND	+	+
DNA G + C content (mol%)	30.0	31.0	40.6	38.0	32.5

strains could be readily distinguished from related species of the genus *Flavobacterium* on the basis of several differential phenotypic properties, such as the absence of oxidase and catalase activities, an inability to hydrolyse starch and the ability to hydrolyse Tween 80.

Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987). For quantitative analysis of the cellular fatty acid compositions of strains LW30^T and LW29, 40 mg wet cell material was harvested from R2A agar plates after 24 h of incubation at 30 °C and the cellular fatty acids were extracted, saponified and methylated according to the protocol of the Sherlock Microbial Identification System (MIDI). Fatty acids were analysed by GC (6890; Hewlett Packard) and identified by the Microbial Identification software package (Sasser, 1990). Chromosomal DNA was isolated and purified using a Cell Culture DNA Midi kit (Qiagen) according to the manufacturer's protocol and the G + C content of the DNA was determined by using the fluorescence monitoring method (Xu *et al.*, 2000; Gonzalez & Saiz-Jimenez, 2002) with a real-time thermal cycler (Mx3000p; Stratagene). DNA of *Escherichia coli* strain B (Sigma-Aldrich) was used as the calibration reference.

The major respiratory quinone was MK-6, as in all members of the family *Flavobacteriaceae*. The cellular fatty acid profiles of strains LW30^T and LW29 and related species are presented in Supplementary Table S1, available in IJSEM Online. Strains LW30^T and LW29 contained large amounts of iso-C_{15:0} (21.3–21.6 %), C_{15:0} (12.2–12.3 %), iso-C_{15:1} G (10.5–10.6 %) and iso-C_{17:1}ω9c (9.8–10.5 %) and smaller amounts of iso-C_{17:0} 3-OH (6.6–7.2 %), iso-C_{15:0} 3-OH (6.4–6.6 %), iso-C_{16:0} (5.3–5.4 %), an unknown fatty acid of estimated chain length (ECL) 13.565 (4.5–4.6 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 2.8 %), C_{16:0} (2.3–2.5 %), anteiso-C_{15:0} (1.8–1.9 %), an unknown fatty acid of ECL 11.543 (1.7–2.1 %), iso-C_{16:1} H (1.1–1.3 %), C_{15:1}ω6c (1.0–1.1 %), C_{17:1}ω6c (1.1–1.2 %), C_{15:0} 3-OH (1.1–1.2 %), C_{16:0} 3-OH (1.1–1.4 %) and C_{17:0} 3-OH (1.0–1.1 %). These data are in agreement with data previously reported for other members of the genus *Flavobacterium* (Bernardet & Bowman, 2006). The DNA G + C content of both strains LW30^T and LW29 was 30 mol%, which is within the range recognized for species of the genus *Flavobacterium* (30–41 mol%; Bernardet & Bowman, 2006).

The 16S rRNA genes of strains LW30^T and LW29 were amplified by PCR using the universal primer set 9F and

1512R as described previously (Quan *et al.*, 2005). The PCR product was purified with a QIAquick PCR purification kit (Qiagen) and sequenced using an Applied Biosystems 3730XL DNA analyser and primers 519F, 536R, 907F and 1100R (Quan *et al.*, 2005). The 16S rRNA gene sequences of type strains of related taxa were obtained from EzTaxon (Chun *et al.*, 2007) and multiple alignments were performed using the CLUSTAL_X program (Thompson *et al.*, 1997). Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Phylogenetic trees were constructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 1993) algorithms using the MEGA4 program (Tamura *et al.*, 2007), with bootstrap values based on 1000 replications (Felsenstein, 1985). Evolutionary distances were calculated using the method of Jukes & Cantor (1969).

The nearly complete 16S rRNA gene sequences of strains LW30^T (1422 bp) and LW29 (1421 bp) were obtained. Strain LW29 showed 99.5% sequence similarity to strain LW30^T. Phylogenetic trees based on the neighbour-joining and maximum-parsimony methods indicated that the novel strains, together with *Flavobacterium indicium* GPTSA 100-9^T, formed a separate clade within the genus *Flavobacterium* (Fig. 1). On the basis of 16S rRNA gene sequence similarity, the closest relatives were *Flavobacterium cheniae* NJ-26^T (94.0%) and *F. indicium* GPTSA 100-9^T (93.9%), suggesting that strains LW30^T and LW29 represent a novel species of the genus *Flavobacterium*.

On the basis of the phylogenetic tree and fatty acid profiles, it is proposed that strains LW30^T and LW29 belong to the genus *Flavobacterium*. Based on the phylogenetic distance and phenotypic differences between these strains and the type strains of other members of the genus *Flavobacterium*, strains LW30^T and LW29 represent a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium dongtanense* sp. nov. is proposed.

Description of *Flavobacterium dongtanense* sp. nov.

Flavobacterium dongtanense (dong.tan.en'se. N.L. neut. adj. *dongtanense* pertaining to Dongtan, a wetland region in Chongming Island, Shanghai, China, from where the type strain was isolated).

Cells are Gram-reaction-negative, aerobic, non-spore-forming rods, approximately 0.4–0.5 × 1.0–2.2 µm. Colonies on R2A are translucent, pale-yellow, circular and 1–2 mm in diameter after 2 days of incubation at 30 °C. Cells do not produce flexirubin-type pigments on R2A. Growth occurs at 4–37 °C (optimum 30 °C), but not at 42 °C, at pH 6–9 (optimum pH 7–8) and in the presence of 0–3% NaCl (optimum 0–1%), but not in the presence of 5% NaCl. Catalase- and oxidase-negative. Positive for esterase lipase (C8), alkaline phosphatase, acid phosphatase and valine arylamidase activities but negative for urease, α- and β-glucosidase, lipase (C14), arginine dihydrolase, α-chymotrypsin, α- and β-galactosidase, naphthol-AS-BI-phosphohydrolase, esterase (C4), leucine arylamidase, cystine arylamidase, β-glucuronidase, α-fucosidase, α-mannosidase and N-acetyl-β-glucosaminidase activities. Positive for hydrolysis of gelatin and Tween 80 but negative for glucose acidification, indole production and hydrolysis of starch, aesculin and trypsin. Nitrate is not reduced. The following substrates are not utilized as sole carbon sources: adipate, arabinose, caprate, malate, citrate, glucose, gluconate, maltose, mannitol, mannose, N-acetylglucosamine and phenylacetate. The major respiratory lipoquinone is menaquinone-6 (MK-6). The major fatty acids are iso-C_{15:0}, C_{15:0}, iso-C_{15:1} G and iso-C_{17:1}ω9c, with smaller amounts of iso-C_{17:0} 3-OH, iso-C_{15:0} 3-OH, iso-C_{16:0}, unknown fatty acid of ECL 13.565, summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), C_{16:0}, anteiso-C_{15:0}, unknown fatty acid of ECL 11.543, iso-C_{16:1} H, C_{15:1}ω6c, C_{17:1}ω6c, C_{15:0} 3-OH, C_{16:0} 3-OH and C_{17:0} 3-OH. The genomic DNA G + C content of the type strain is 30 mol%.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strains LW30^T and LW29 and related members of the genus *Flavobacterium*. Filled circles indicate branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) greater than 50 are shown at the branching points. Bar, 0.01 substitutions per nucleotide position.

The type strain, LW30^T (=KCTC 22671^T =CCTCC AB 209201^T) and strain LW29 (=KCTC 22670 =CCTCC AB 209200) were isolated from the rhizosphere of a wetland reed in Dongtan, Chongming Island, Shanghai, China.

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