Flavobacterium cheniae sp. nov., isolated from sediment of a eutrophic reservoir

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A Gram-negative, rod-shaped, yellow pigmented bacterium, strain NJ-26^T, was isolated from sediment of the eutrophicated Guanting Reservoir in Beijing, China. A phylogenetic analysis based on 16S rRNA gene sequences placed strain NJ-26^T within the genus *Flavobacterium* in the family *Flavobacteriaceae*. The highest sequence similarity was found with *Flavobacterium cucumis* R2A45-3^T (97.7 %). The major fatty acids (>5 %) of the isolate were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, $C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G and iso-$C_{15:0}$ 3-OH. The G+C content of the genomic DNA was 40.6 mol%. The DNA–DNA relatedness value with *F. cucumis* R2A45-3^T was 5.4 %. Molecular and phenotypic data suggest that strain NJ-26^T represents a novel species within the genus *Flavobacterium*, for which the name *Flavobacterium cheniae* sp. nov. is proposed. The type strain is NJ-26^T (=CGMCC 1.6844^T =NBRC 103934^T).

The genus *Flavobacterium*, belonging to the phylum *Bacteroidetes* (formerly the *Cytophaga–Flavobacterium–Bacteroides* group), was proposed by Bergey *et al.* (1923) and its description was considerably emended by Bernardet *et al.* (1996). *Flavobacterium* species have been isolated from a wide range of habitats (sediment, fresh water, seawater, soil, micromats, etc.) and display a variety of physiological characteristics (Bernardet & Bowman, 2006). Recently described species are *Flavobacterium defluvii*, *F. aquidurense*, *F. hercynium*, *F. terrigena*, *F. terrae* and *F. cucumis* (Park *et al.*, 2007; Cousin *et al.*, 2007; Yoon *et al.*, 2007; Weon *et al.*, 2007). In the present study, a novel species is proposed following the polyphasic taxonomy study of a sediment isolate.

A sediment sample was collected in July 2006 from the eutrophicated Guanting Reservoir in Beijing city, China. Characteristics of the sample were: pH 7.7, 276.54 mg microbial biomass carbon kg^{-1}, 3.92 % organic carbon, 0.48 % total nitrogen, 0.04 % available K and 8.2 mg available P kg^{-1}.

For isolation, the sediment sample was suspended in sterilized water, serially diluted and spread on plates of sediment steep medium prepared as follows: 400 g sediment was boiled in 1 l distilled water for 10 min and then 0.5 g peptone, 0.5 g yeast extract and 15 g agar were added to 1 l supernatant; the pH was not adjusted. Plates were incubated at 28 °C for 10 days. A single colony of strain NJ-26^T was picked and routinely subcultured on modified R2A medium (yeast extract, 0.5 g; polypeptone, 0.5 g; Casamino acids, 0.5 g; glucose, 0.5 g; soluble starch, 0.5 g; K_2HPO_4, 0.3 g; $MgSO_4 . 7H_2O$, 0.05 g; distilled water 1 l; pH 7.2).

The 16S rRNA gene of strain NJ-26^T was amplified by PCR using the universal bacterial primers 27F and 1495R (Ying

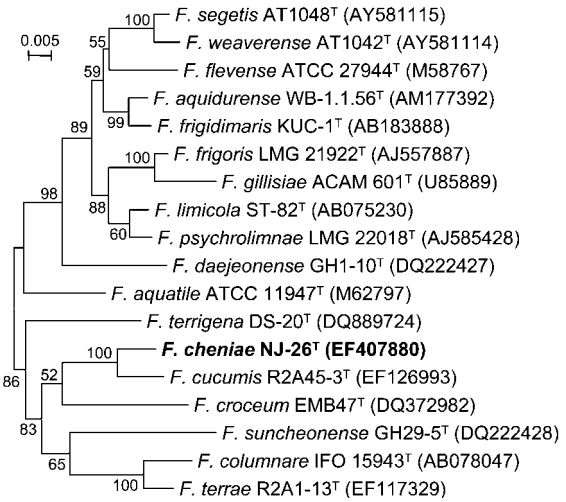

Fig. 1. Neighbour-joining tree based on the 16S rRNA gene sequences of strain NJ-26^T and members of closely related *Flavobacterium* species. Numbers at nodes are bootstrap percentages >50 % from 1000 replicates. Bar, 0.005 substitutions per nucleotide position. Minimum-evolution and maximum-parsimony trees are shown in Supplementary Fig. S1.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NJ-26^T is EF407880.

Maximum-parsimony and minimum-evolution phylogenetic trees based on 16S rRNA gene sequences are available as supplementary material with the online version of this paper.

et al., 2007) and the 1410 bp amplified fragment was sequenced with an ABI 3730 XL 96-capillary sequencer (Applied Biosystems). The search for phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). Phylogenetic trees were constructed by using the neighbour-joining, maximum-parsimony and minimum-evolution algorithms available in MEGA version 3.1 (Kumar *et al.*, 2004) after multiple alignment of the data by CLUSTAL W (Thompson *et al.*, 1994). The topology of the tree was evaluated by using Kimura's two-parameter calculation model (Kumar *et al.*, 2004) based on 1000 replicates. In the neighbour-joining phylogenetic tree (Fig. 1), the isolate was located within the genus *Flavobacterium*, sharing 97.7 % 16S rRNA sequence similarity with *F. cucumis* R2A45-3^T (Weon *et al.*, 2007) (bootstrap value 100 %). It was also closely related to *F. croceum* EMB47^T (Park *et al.*, 2006), *F. suncheonense* DSM 17707^T (Kim *et al.*, 2006), *F. columnare* IFO 15943^T (Bernardet *et al.*, 1996), *F. terrae* R2A1-13^T (Weon *et al.*, 2007), *F. terrigena* DS-20^T (Yoon *et al.*, 2007) and *F. aquatile* ATCC 11947^T (Bernardet *et al.*, 1996), with sequence similarities of 93.7–95.1 %. The same general topology was found in the maximum-parsimony and minimum-evolution phylogenetic trees, available as Supplementary Fig. S1 in IJSEM Online.

Growth at 4, 18, 22, 28, 32, 37 and 50 °C and pH 5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 was assessed after 10 days of incubation. The pH of R2A broth was adjusted by adding 1 M NaOH or 1 M HCl. Tolerance to salinity was tested in R2A broth supplemented with 0–5.0 % NaCl (w/v) at 1 % intervals after 10 days of incubation. Growth on nutrient agar (NA), trypticase soy agar (TSA), marine agar 2216 (MA) and PYG agar was also evaluated at 28 °C for 10 days. All media were prepared in the laboratory following the composition of Difco media (NA, TSA and MA) or according to Zhu *et al.* (2003) (PYG). Flexirubin-type pigments were sought by flooding a small mass of bacterial cells with 20 % KOH according to Bernardet *et al.* (2002). Motility was investigated using a fresh R2A broth culture using the hanging drop technique. Hydrolysis of

Table 1. Differential characteristics of strain NJ-26^T and related *Flavobacterium* species

Taxa: 1, strain NJ-26^T (*Flavobacterium cheniae* sp. nov.); 2, *F. cucumis* R2A45-3^T (data from Weon *et al.*, 2007); 3, *F. croceum* EMB47^T (Park *et al.*, 2006); 4, *F. suncheonense* DSM 17707^T (Kim *et al.*, 2006); 5, *F. columnare* IFO 15943^T (Bernardet & Grimont, 1989; Bernardet *et al.*, 1996); 6, *F. terrae* R2A1-13^T (Weon *et al.*, 2007); 7, *F. terrigena* DS-20^T (Yoon *et al.*, 2007); 8, *F. aquatile* (Bernardet *et al.*, 1996). +, Positive; −, negative; W, weakly positive; NR, not reported; V, variable among references. All strains are positive for oxidase activity and gelatin degradation. All strains tested are negative for degradation of CM-cellulose, chitin and urea, reduction of nitrate and β-galactosidase activity.

Characteristic	1	2	3	4	5	6	7	8
Colony pigmentation*	Y	Y	Y	Y	GY	YO	DY	CY
Growth on:								
NA	+	+	−	+	−	+	+	−
TSA	W	W	W	W	−	W	−	+
Growth at 37 °C	−	+	+	+	−	+	−	+
pH for growth								
Range	6.5–8.0	6.0–8.0	5.5–8.5	6–8	NR	6.0–8.0	6.0–8.0	NR
Optimum	7.5	7.0	7.5–8.0	NR	NR	7.0	6.5–7.0	NR
NaCl concentration for growth (%)								
Range	0–0.8	0–2.0	0–1.0	0–1.0	0–0.5	0–2.0	NR	<0.5
Optimum	0	0–2.0	0	NR	0	0–2.0	NR	0
Catalase activity	+	+	−	+	+	−	+	+
Flexirubin reaction	−	−	−	−	+	+	+	−
Gliding motility	+	+	−	−	+	−	−	+
Glucose utilization	−	−	+	−	−	−	−	NR
Acid from carbohydrates	−	−	+	−	−	−	−	+
Degradation of:								
Starch	W	+	−	−	−	+	−	V
Casein	−	+	+	+	+	+	+	+
Tyrosine	+	+	−	+	−	−	+	V
DNA	−	−	NR	−	+	−	−	−
H_2S production	W	NR	−	NR	+	NR	−	−
Brown pigment on tyrosine agar	+	NR	NR	NR	−	NR	NR	−
Precipitation on egg-yolk agar	+	NR	NR	+	+	NR	NR	+
DNA G+C content (mol%)	40.6	38	40.8	39	32	34	38.2	33

*CY, Cream–yellow; DY, dark yellow; GY, greenish yellow; Y, yellow; YO, yellowish orange.

pectin, chitin, Tween 80, CM-cellulose, casein, DNA and urea was investigated as described by Park *et al.* (2006) or Dong & Cai (2001). Utilization of carbon and energy sources was investigated in a basal medium containing (l^{-1}) 1 g $(NH_4)_2HPO_4$, 0.1 g yeast extract, 0.3 g K_2HPO_4, 0.05 g $MgSO_4.7H_2O$, pH 7.2. Carbon substrates were added at a concentration of 0.5 % (w/v). The following biochemical and physiological tests were performed according to Dong & Cai (2001) or Kim *et al.* (2006): oxidase and catalase activities, formation of a precipitate on egg-yolk agar, production of acid from carbohydrates, hydrolysis of tyrosine and production of a brown pigment on tyrosine agar, glucose utilization, nitrate reduction, production of H_2S, alkaline phosphatase, lipase, β-galactosidase, urease, tryptophan deaminase and arginine dihydrolase activities and hydrolysis of gelatin, starch and *o*-nitrophenyl β-D-galactopyranoside (ONPG).

The phenotypic characteristics of strain NJ-26^T are given in Table 1 and in the species description. Strain NJ-26^T could be distinguished from its closest phylogenetic neighbour, *F. cucumis* R2A45-3^T, by the hydrolysis of casein, ranges of temperature and pH for growth, tolerance to NaCl and optimum NaCl concentration.

Cells grown on R2A agar for 2 days at 28 °C were used for cellular fatty acid analysis. Fatty acid methyl esters were prepared and identified using the Sherlock Microbial Identification System (MIDI). The major cellular fatty acids (>5 %) of strain NJ-26^T were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, $C_{15:0}$, iso-$C_{17:0}$ 3-OH, iso-$C_{15:1}$ G and iso-$C_{15:0}$ 3-OH. The proportion of several fatty acids clearly distinguished strain NJ-26^T from *F. cucumis* R2A45-3^T (Table 2). However, these discrepancies may result partly from the use of different culture conditions.

Genomic DNA was extracted according to Marmur (1961) and Johnson (1985a, b) from cells cultured for 2 days in R2A broth. The G+C content of the DNA was determined by thermal denaturation (De Ley, 1970) using DNA from *Escherichia coli* K-12 as a control. The DNA G+C content

Table 2. Cellular fatty acid compositions of strain NJ-26^T and type strains of related *Flavobacterium* species

Strains: 1, strain NJ-26^T (*F. cheniae* sp. nov.); 2, *F. cucumis* R2A45-3^T (data from Weon *et al.*, 2007); 3, *F. croceum* EMB47^T (Park *et al.*, 2006); 4, *F. suncheonense* DSM 17707^T (Kim *et al.*, 2006); 5, *F. columnare* IFO 15943^T (Weon *et al.*, 2007); 6, *F. terrae* R2A1-13^T (Weon *et al.*, 2007); 7, *F. terrigena* DS-20^T (Yoon *et al.*, 2007); 8, *F. aquatile* DSM 1132^T (Weon *et al.*, 2007). Fatty acids amounting to <1 % in all strains studied are not listed. Some of the strains listed were grown under different culture conditions. tr, Traces (<1 %).

Fatty acid	1	2	3	4	5	6	7	8
iso-$C_{13:0}$	tr		1.0	1.9	1.3			
iso-$C_{14:0}$		5.3	8.7	tr	3.8	4.7		3.1
iso-$C_{14:0}$ 3-OH		1.8	2.6		1.5	1.1		
Unknown 13.565	1.6							
$C_{15:0}$	15.4		10.8			4.3		
$C_{15:0}$ 2-OH			2.0	tr				
$C_{15:0}$ 3-OH	1.4	1.5	1.3	tr	1.9	2.2		2.1
anteiso-$C_{15:0}$	2.0	4.4	5.1	2.1	2.2	2.8		3.5
iso-$C_{15:0}$	27.7	17.5	9.3	29.9	30.1	18.4	30.1	17.0
iso-$C_{15:0}$ 3-OH	6.3	5.1	5.9	11.1	5.2	6.7	6.6	6.4
iso-$C_{15:1}$ G	7.2	9.9	11.5	12.0	14.6	10.7		7.4
iso-$C_{15:1}$							9.0	
$C_{15:1}\omega6c$	2.7	2.0		tr		1.1	1.3	10.8
anteiso-$C_{15:1}$ A			1.6					
$C_{16:0}$	tr	1.2	1.0	1.0	1.1			2.6
$C_{16:0}$ 3-OH	tr	1.7	tr			1.3		2.3
iso-$C_{16:0}$	tr	18.8	8.5	1.0	8.7	16.6	5.2	7.2
iso-$C_{16:0}$ 3-OH	tr	12.6	16.5	tr	7.4	5.0	3.6	10.4
iso-$C_{16:1}$		4.0	2.9		4.1	3.0	2.2	2.9
$C_{17:0}$ 3-OH	tr		tr	tr		1.5		1.1
iso-$C_{17:0}$ 3-OH	7.6	5.3	2.7	17.7	6.6	10.3	16.0	6.5
$C_{17:1}\omega6c$	tr							2.5
$C_{17:1}\omega8c$	tr							1.3
iso-$C_{17:1}\omega9c$	15.5	1.5		7.5	3.7	6.2	11.7	1.5
$C_{18:1}\omega5c$	1.5			tr				
Summed feature 3*	2.5	2.0	3.6	9.8		1.3	2.4	7.0

*Summed features are combinations of fatty acids that cannot be separated by the MIDI system. Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

of the isolate was 40.6 mol%, within the range of 30–42 mol% reported in the genus *Flavobacterium* (Bernardet & Bowman, 2006).

Genomic relatedness between the isolate and its most closely related phylogenetic neighbour *F. cucumis* R2A45-3^T (obtained from Dr Soon-Wo Kwon, KACC, Suwon, Korea) was determined by DNA–DNA hybridization, which was carried out spectrophotometrically (De Ley, 1970). The hybridization value was 5.4 %, indicating that the isolate represents a different species from *F. cucumis* R2A45-3^T.

The results of a polyphasic taxonomy study support the description of strain NJ-26^T as a representative of a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium cheniae* sp. nov. is proposed.

Description of *Flavobacterium cheniae* sp. nov.

Flavobacterium cheniae (che′ni.ae. N.L. fem. gen. n. *cheniae* of Chen, named in honour of Professor Wen-Xin Chen, for her contribution to environmental microbiology).

Cells are aerobic, Gram-negative, rods, 0.3–0.5 μm in diameter and 1.5–3.0 μm long, motile by gliding. Colonies on R2A agar incubated at 28 °C for 2 days are yellow, circular, about 1 mm in diameter and non-spreading. Growth occurs at 18–32 °C (optimum, 28 °C), at pH 6.5–8.0 (optimum, 7.5) and with 0–0.8 % NaCl (optimum growth in the absence of NaCl). Growth occurs on NA and TSA (weakly), but not on MA or PYG agar. Oxidase- and catalase-positive. Flexirubin-type pigments are absent. H_2S is produced weakly. Nitrate is not reduced. ONPG is not hydrolysed. Gelatin is degraded strongly, but pectin, chitin, Tween 80, CM-cellulose, casein, DNA and urea are not. Tyrosine is degraded and a brown pigment is produced on tyrosine agar. Alkaline phosphatase and weak lipase activities are present, but β-galactosidase, urease, tryptophan deaminase and arginine dihydrolase activities are absent. Does not assimilate any of the carbohydrates tested, including raffinose, salicin, lactose, melezitose, D-mannose, trehalose, melibiose, glucose, sucrose, maltose, mannitol, D-galactose, L-rhamnose, D-xylose, D-sorbose, D-sorbitol, D-fructose, inulin, D-arabinose, L-arabinose, D-ribose, inositol, citrate, sorbic acid, succinate, glycerol and aspartic acid. The major cellular fatty acids (>5 %) of the type strain are iso-$C_{15:0}$ (27.7 %), iso-$C_{17:1}\omega 9c$ (15.5 %), $C_{15:0}$ (15.4 %), iso-$C_{17:0}$ 3-OH (7.6 %), iso-$C_{15:1}$ G (7.2 %) and iso-$C_{15:0}$ 3-OH (6.3 %). The DNA G+C content of the type strain is 40.6 mol%.

The type strain is NJ-26^T (=CGMCC 1.6844^T =NBRC 103934^T), isolated from sediment of Guanting Reservoir in Beijing, China.

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