Flavitalea populi gen. nov., sp. nov., isolated from soil of a Euphrates poplar (*Populus euphratica*) forest

Yang Wang,¹ Feng Cai,¹ Yali Tang,² Jun Dai,¹ Huan Qi,¹ Erkin Rahman,³ Fang Peng¹ and Chengxiang Fang¹

Correspondence

Chengxiang Fang

cxfang@whu.edu.cn

Fang Peng

fangpeng2@yahoo.com.cn

¹China Center for Type Culture Collection (CCTCC), College of Life Sciences, Wuhan University, Wuhan 430072, PR China

²Institute of Hydrobiology, Jinan University, Guangzhou 510632, PR China

³College of Life Science and Technology, Xinjiang University, Urumchi 830046, PR China

A novel strain, designated HY-50R^T, isolated from soil of a Euphrates poplar (*Populus euphratica*) forest in Xinjiang, China, was characterized using a polyphasic taxonomic approach. Cells of the isolate were Gram-reaction-negative, strictly aerobic, rod-shaped, non-motile, oxidase-negative and catalase-positive. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate was a member of the phylum *Bacteroidetes*, its closest relatives being *Niastella populi* THYL-44^T (93.6 % similarity), *Flavisolibacter ginsengisoli* Gsoil 643^T (93.5 %), *Terrimonas ferruginea* IAM 15098^T (93.3 %) and *Flavisolibacter ginsengiterrae* Gsoil 492^T (93.2 %). The major fatty acids were iso-C_{15:1} G (11.7 %), iso-C_{15:0} (19.6 %) and iso-C_{17:0} 3-OH (19.3 %). The predominant menaquinone of strain HY-50R^T was MK-7 and the genomic DNA G + C content was 46.8 mol%. Flexirubin-type pigments were not produced. Based on phylogenetic evidence and the results of phenotypic, genotypic and chemotaxonomic analysis, strain HY-50R^T represents a novel species of a novel genus, for which the name *Flavitalea populi* gen. nov., sp. nov. is proposed. The type strain is HY-50R^T (=CCTCC AB 208255^T =NRRL B-59222^T).

The phylum *Bacteroidetes* (Garrity & Holt, 2001) is phenotypically diverse and is still undergoing considerable taxonomic expansion and organization. Recently, the family *Chitinophagaceae*, belonging to the phylum *Bacteroidetes*, was established to incorporate several genera on the basis of 16S rRNA gene sequence analyses and some phenotypic similarities (Kämpfer *et al.*, 2011). The family *Chitinophagaceae* encompasses 15 genera, *Balneola* (Urios *et al.*, 2006), *Chitinophaga* (Kämpfer *et al.*, 2006), *Ferruginibacter* (Lim *et al.*, 2009), *Filimonas* (Shiratori *et al.*, 2009), *Flaviumibacter* (Zhang *et al.*, 2010), *Flavisolibacter* (Yoon & Im, 2007), *Gracilimonas* (Choi *et al.*, 2009), *Hydrotalea* (Kämpfer *et al.*, 2011), *Lacibacter* (Qu *et al.*, 2009), *Niabella* (Kim *et al.*, 2007), *Niastella* (Weon *et al.*, 2006), *Parasegetibacter* (Zhang *et al.*, 2009), *Sediminibacterium* (Qu & Yuan, 2008), *Segetibacter* (An *et al.*, 2007) and *Terrimonas* (Xie & Yokota, 2006). During the course of

a study on the diversity of bacterial communities associated with soil from a Euphrates poplar forest in Xinjiang, China, a bacterial strain, designated HY-50R^T, which formed yellow-pigmented colonies, was isolated. Comparative analysis of 16S rRNA gene sequences suggested that strain HY-50R^T was most closely related to members of the family *Chitinophagaceae* within the phylum *Bacteroidetes*. The strain was, therefore, subjected to further taxonomic investigation, the results of which suggest that strain HY-50R^T represents a novel species of a novel genus in the family *Chitinophagaceae*.

To isolate the strain, a soil sample was serially diluted with sterilized water and the dilutions were plated onto R2A agar (Difco) and incubated at 30 °C for 4 days. Growth of strain HY-50R^T was also evaluated on nutrient agar (NA, Difco), tryptic soy agar (TSA, Difco), 0.1 × TSA and MacConkey agar (Difco) at 30 °C. As strain HY-50R^T grew well on NA, R2A, TSA and 0.1 × TSA, it was routinely cultured on 0.1 × TSA at 30 °C.

Colony morphology was observed visually and cell morphology and motility were observed using phase-contrast

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY-50R^T is HM130561.

One supplementary figure is available with the online version of this paper.

microscopy (Olympus BX51) using two-day-old cultures grown on 0.1 × TSA at 30 °C. Gram-staining was performed using the modified Hucker method (Gerhardt *et al.*, 1994). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by measuring bubble production after the addition of 3 % (v/v) hydrogen peroxide solution. Tests for the hydrolysis of starch (1 %, w/v), cellulose (0.1 %, w/v), chitin from crab shells (1 %, w/v) and casein (5 %, w/v) were carried out as described by Smibert & Krieg (1994). Growth under anaerobic conditions was determined by incubation at 30 °C on 0.1 × TSA for 10 days in a GasPak (BBL) jar. Growth at 4, 10, 15, 30, 37 and 42 °C was tested on 0.1 × TSA. Growth at different pH levels was determined on 0.1 × TSA adjusted to pH 4–10 at intervals of 1 pH unit before sterilization (Xu *et al.*, 2005). Tolerance of NaCl was determined on 0.1 × TSA supplemented with 1–5 % (w/v) NaCl at intervals of 1 %. The presence of flexirubin-type pigments was tested spectrophotometrically as described previously (Güde, 1980) using 20 % KOH. Enzyme activities and biochemical characteristics were determined by using API 20 NE, API 20 E, API ZYM and API ID 32 GN test kits (bioMérieux) according to the manufacturer's instructions.

Menaquinones were extracted from lyophilized cells and identified using the method described by Xie & Yokota (2003) using HPLC (UltiMate 3000, Dionex). Polyamines were analysed as described previously (Schenkel *et al.*, 1995). The cellular fatty acids of strain HY-50R^T and

closely related type strains were saponified, methylated and extracted from cells grown on R2A at 30 °C, according to the protocol of the Sherlock microbial identification system (MIDI, 1999), and analysed using GC (Agilent 6890).

Genomic DNA was prepared according to the method of Wilson (1987). The DNA G + C content was determined by HPLC (Mesbah *et al.*, 1989). The 16S rRNA gene of strain HY-50R^T was amplified by PCR using an established method (Lane, 1991) and the product was sequenced (Invitrogen). 16S rRNA gene sequences of closely related species were retrieved from the EzTaxon database (Chun *et al.*, 2007) and the sequences were aligned with the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic analysis of 16S rRNA gene sequences was performed using MEGA version 4.0 software (Tamura *et al.*, 2007). Phylogenetic distances were calculated according to the neighbour-joining method (Saitou & Nei, 1987), using the Kimura two-parameter model (Kimura, 1980), and the maximum-parsimony method (Fitch, 1971), using the close-neighbour-interchange algorithm (search level=2, random additions=100). Each resultant tree was evaluated by bootstrap analysis on the basis of 1000 replications (Felsenstein, 1985).

The almost-complete 16S rRNA gene sequence of strain HY-50R^T, comprising a continuous stretch of 1434 nt, was determined and compared with sequences of representatives of the phylum *Bacteroidetes*. Sequence similarity calculations performed after a neighbour-joining analysis

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain HY-50R^T and representative members of the phylum *Bacteroidetes*. The tree was based on a comparison of 16S rRNA gene sequences that were at least 90 % complete. Bootstrap values >50 % (based on 1000 replications) are given at branch points. Bar, 0.01 substitutions per nucleotide position.

Table 1. Phenotypic characteristics of strain HY-50R^T and representatives of related genera of the family *Chitinophagaceae*

Taxa: 1, strain HY-50R^T; 2, *Ferruginibacter*; 3, *Filimonas*; 4, *Flaviumibacter*; 5, *Flavisolibacterium*; 6, *Hydrotalea*; 7, *Lacibacter*; 8, *Niabella*; 9, *Niastella*; 10, *Parasegetibacter*; 11, *Sediminibacterium*; 12, *Segetibacter*; 13, *Terrimonas*. Data in columns 4 and 6 from Zhang *et al.* (2010) and Kämpfer *et al.* (2011), respectively. All other data are from this study except for that of the type strain *Terrimonas aquatica* RIB1-6^T (Sheu *et al.*, 2010). +, All species positive; –, all species negative; w, all species weakly positive; v, variable among species; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Gliding motility	–	–	+	–	–	–	+	–	v	+	+	–	–
Gram-reaction	–	–	–	+	–	–	–	–	–	–	–	–	–
Growth at 37 °C	+	–	–	+	v	+	+	–	v	+	+	–	+
Hydrolysis of:													
Chitin	–	–	–	ND	–	ND	–	–	v	–	–	–	–
Casein	–	–	–	+	–	ND	+	v	+	–	–	–	v
Starch	–	–	–	–	–	ND	+	v	–	+	–	–	v
Gelatin	–	+	+	+	+	ND	+	v	+	+	+	–	v
L-Arginine	–	–	–	+	–	ND	–	–	–	–	–	–	–
Production of:													
Flexirubin-type pigments	–	–	–	ND	–	–	–	+	–	–	–	–	v
Indole	–	–	–	ND	–	ND	–	v	–	–	–	–	–
Oxidase	–	+	+	w	v	+	+	v	v	+	+	+	+
Catalase	+	+	+	+	v	+	+	+	–	+	+	+	+
β-Galactosidase	+	v	+	ND	+	+	+	+	+	+	+	–	+
Assimilation of:													
D-Glucose	–	–	+	ND	–	–	–	+	v	+	–	–	+
L-Arabinose	–	–	+	+	v	–	–	+	v	w	–	–	+
D-Mannose	–	–	+	+	v	–	–	+	v	+	–	–	v
D-Mannitol	–	–	–	–	–	–	–	v	–	–	–	–	–
N-Acetylglucosamine	–	–	–	ND	v	–	+	+	–	+	–	–	v
Maltose	–	–	+	+	v	–	+	+	v	+	–	–	+
Potassium gluconate	–	–	–	–	v	–	–	v	–	–	–	–	–
Trisodium citrate	–	–	–	ND	–	–	–	v	–	–	–	–	–

(Fig. 1) indicated that strain HY-50R^T belonged to the family *Chitinophagaceae*. The closest relatives of strain HY-50R^T were *Niastella populi* THYL-44^T (93.6 % 16S rRNA gene sequence similarity), *Flavisolibacter ginsengisoli* Gsoil 643^T (93.5 %), *Terrimonas ferruginea* IAM 15098^T (93.3 %) and *Flavisolibacter ginsengiterrae* Gsoil 492^T (93.2 %). The phylogenetic tree reconstructed using the maximum-parsimony method (Supplementary Fig. S1, available in IJSEM Online) was similar to that reconstructed using the neighbour-joining method. Analysis of the DNA showed that the G+C content of strain HY-50R^T was 46.8 mol%.

Strain HY-50R^T was oxidase-negative and catalase-positive. Cells of the isolate were Gram-reaction-negative and 0.2–0.4 × 1.7–2 µm. Growth of the strain occurred at 15–37 °C, at pH 5–8 and in 0–2 % (w/v) NaCl. Yellow-coloured flexirubin-like pigment was not produced. Other phenotypic features of strain HY-50R^T are summarized in the species description. Strain HY-50R^T exhibited some phenotypic differences when compared with other representatives of the family *Chitinophagaceae* (Table 1).

Furthermore, several characteristics, such as the inability to degrade gelatin and the presence of α-mannosidase activity, distinguished strain HY-50R^T from its closest phylogenetic neighbours. Differential characteristics of strain HY-50R^T and closely related type strains are shown in Table 2.

Menaquinone 7 (MK-7) was detected as the major respiratory quinone of strain HY-50R^T, which is characteristic of species of the family *Chitinophagaceae*. The polyamine pattern showed a predominance of homospermidine. Strain HY-50R^T possessed iso-C_{15:1} G (11.7 %), iso-C_{15:0} (19.6 %) and iso-C_{17:0} 3-OH (19.3 %) as the major fatty acids. In addition, iso-C_{13:0} (6.1 %), summed feature 1 (C_{13:0} 3-OH and/or iso-C_{15:1} H, 5.6 %) and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7c, 6.9 %) were produced in moderate amounts. Fatty acid profiles of strain HY-50R^T and the type strains of related species are shown in Table 3. The presence of a relatively high proportion of iso-C_{13:0} (6.1 %) distinguished strain HY-50R^T from recognized species of phylogenetically related genera. Strain HY-50R^T could also be differentiated

Table 2. Phenotypic characteristics of strain HY-50R^T and closely related type strains of members of the family Chitinophagaceae

Taxa: 1, strain HY-50R^T; 2, *Flavisolibacterium ginsengisoli* Gsoil 643^T; 3, *Flavisolibacterium ginsengiterrae* Gsoil 492^T; 4, *Niastella populi* THYL-44^T; 5, *Terrimonas ferruginea* DSM 30193^T. All data from this study. +, Positive; –, negative; w, weakly positive.

Characteristic	1	2	3	4	5
Growth on TSA	+	–	–	+	+
Tolerance of NaCl (% w/v)	2.0	1.0	3.0	1.0	1.0
Enzyme activities:					
Esterase (C4), esterase lipase (C8)	+	+	+	–	+
α-Chymotrypsin	–	+	+	–	–
α-Galactosidase	+	+	+	–	–
α-Glucosidase	+	+	+	–	w
β-Glucosidase	+	w	+	–	–
α-Mannosidase	+	–	–	–	–
Assimilation of D-Glucose, L-arabinose, D-mannose, maltose	–	–	–	+	+

from the type species of its closest relatives by the absence of iso-C_{15:0} 3-OH and C_{18:0} and by some other quantitative differences between fatty acid compositions.

Based on the phenotypic, chemotaxonomic and genetic data and the relatively low levels of 16S rRNA gene sequence similarity between strain HY-50R^T and members of related genera of the family Chitinophagaceae, strain HY-50R^T represents a novel species of a novel genus in the family Chitinophagaceae, for which the name *Flavitalea populi* gen. nov., sp. nov. is proposed.

Description of *Flavitalea* gen. nov.

Flavitalea (Fla.vi.ta'le.a. L. adj. *flavus* yellow; L. fem. n. *talea* a slender staff, a rod; N.L. fem. n. *Flavitalea* a yellow-coloured rod).

Cells are Gram-reaction-negative, strictly aerobic, rod-shaped and non-motile. Oxidase-negative and catalase-positive. MK-7 is the major respiratory quinone. Flexirubin-like pigments are not detected. Homospermidine is the major polyamine component. Phylogenetically positioned within the family Chitinophagaceae, phylum Bacteroidetes. The type species is *Flavitalea populi*.

Description of *Flavitalea populi* sp. nov.

Flavitalea populi (po'pu.li. L. gen. n. *populi* of a poplar, pertaining to *Populus*, the genus name of the poplar trees that grow in the forest the strain was isolated from).

Cells are 0.2–0.4 × 1.7–2 µm in size. Colonies are circular, convex, smooth, opaque and yellow on 0.1 × TSA. Grows on NA, R2A, TSA and 0.1 × TSA but not on MacConkey's agar at 30 °C. Grows at 15–37 °C (optimum 30 °C) and pH 5–8 (optimum pH 7). Tolerates up to 2 % (w/v) NaCl. Hydrolyses aesculin but not gelatin, starch, casein, chitin or

cellulose. Nitrate is not reduced to nitrite and hydrogen sulfide is not produced. Positive for *N*-acetyl-D-glucosamine, sucrose, maltose, D-glucose, salicin and melibiose assimilation. Negative for citrate utilization, indole production, glucose acidification, arginine dihydrolase and urease activities and assimilation of L-arabinose, D-mannose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, *myo*-inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, L-fucose, D-sorbitol, propionic acid, valeric acid, L-histidine, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, potassium 2-ketogluconate and L-proline. In the API ZYM system, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-B1-phosphohydrolase, α- and β-galactosidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase activities but negative for lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase and α-fucosidase activities. The major fatty acids are iso-C_{15:1} G, iso-C_{15:0} and iso-C_{17:0} 3-OH.

The type strain, HY-50R^T (=CCTCC AB 208255^T =NRRL B-59222^T), was isolated from a soil sample from a Euphrates poplar forest in Xinjiang, China. The DNA G+C content of type strain is 46.8 mol%.

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Table 3. Cellular fatty acid compositions (%) of strain HY-50R^T and closely related type strains of members of the family *Chitinophagaceae*

Taxa: 1, strain HY-50R^T; 2, *Ferruginibacter alkalilientus* HU1-GD23^T; 3, *Ferruginibacter lapsinans* HU1-HG42^T; 4, *Filimonas lacunae* YT21^T; 5, *Flaviumibacter petaseus* T41^T; 6, *Flavisolibacterium ginsengisoli* Gsoil 643^T; 7, *Flavisolibacterium ginsengiterrae* Gsoil 492^T; 8, *Hydrothalea flava* CCUG 51397^T; 9, *Lacibacter cauensis* NJ-8^T; 10, *Niabella aurantiaca* R2A15-11^T; 11, *Niabella soli* JS13-8^T; 12, *Niabella tibetensis* 15-4^T; 13, *Niabella yanshanensis* CCBAU 05354^T; 14, *Niastella koreensis* GR20-10^T; 15, *Niastella populi* THYL-44^T; 16, *Niastella yeongjuensis* GR20-13^T; 17, *Parasegetibacter luojiensis* RHYL-37^T; 18, *Sediminibacterium salmoneum* NJ-44^T; 19, *Segetibacter koreensis* Gsoil 664^T; 20, *Terrimonas ferruginea* DSM 30193^T; 21, *Terrimonas lutea* DY^T. Data in columns 5 and 8 were taken from Zhang *et al.* (2010) and Kämpfer *et al.* (2011), respectively. Values are percentages of total fatty acids; fatty acids amounting to <1 % of total fatty acids in all strains studied are not listed; tr, trace (<1 %).

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
iso-C _{13:0}	6.1	tr	2.5	tr		tr	tr	4.2	tr	tr		tr		tr	tr	tr	tr	tr	tr	tr	tr
iso-C _{14:0}	tr	tr		tr		tr	2.5		tr			tr	tr	tr	tr	tr	tr	1.3	6.3	tr	tr
C _{14:0}		1.9	1.2	2.4					1.1	1.1	2.7	1.3	1.4			tr	2.1	1.9	1.3	1.6	1.6
iso-C _{13:0} 3-OH	4.8	tr	tr	tr		1.9	1.3		tr		1.5	tr	tr	tr	tr	tr	5.2	1.6	tr	tr	tr
iso-C _{15:1} G	11.7	15.2	16.0	18.7	13.3	5.4	7.0	8.2	10.2	21.8	18.5	13.7	26.4	15.8	17.4	14.8	14.7	17.7		23.7	23.4
iso-C _{15:1} F																			tr		
anteiso-C _{15:1} A	2.9	tr	tr	tr		3.5	5.4		tr	tr	tr	tr	tr	1.0	1.3	tr	3.7	4.4		tr	tr
iso-C _{15:0}	19.6	18.4	32.5	23.7	33.8	24.3	25.7	34.8	33.6	30.6	28.6	42.9	29.0	23.3	28.6	29.1	19.5	17.6	15.3	27.6	20.8
anteiso-C _{15:0}	4.6	2.1	1.3	tr		9.2	9.7	1.7	1.1	1.8	tr	1.3	tr	4.5	4.9	5.5	3.3	10.6	21.1	2.4	1.4
C _{15:0}	1.2	4.2	tr	tr	8.0	1.0	1.6	tr	4.3	8.5	1.6	3.2	1.6	2.7	tr	1.9	2.7	tr	2.9	6.6	5.7
iso-C _{16:1} H						1.1	2.3												3.0		
iso-C _{16:0}	tr	1.2	tr	1.7		1.8	4.2	tr	2.1	tr		tr	2.3	tr	tr	tr	3.0	1.7	13.3	1.0	tr
C _{16:1} ω5c	2.5			1.8	5.8	tr	tr		2.2				2.4			1.1		tr	3.1		
C _{16:0}	2.0	7.7	6.9	4.9	2.2	8.2	4.2	tr	4.4	5.7	9.8	3.9	3.1	4.8	3.9	2.9	10.1	5.0	1.5	5.2	3.5
iso-C _{15:0} 3-OH		11.0	3.3	4.0	3.9	tr	1.1	4.0	3.2	2.5	2.3	2.8	2.8	1.3	1.6	2.1		4.2	1.2	1.8	1.6
C _{15:0} 2-OH	tr	1.4	tr			tr	tr	tr	tr	1.3		tr		1.0	tr	tr	tr	1.2	1.0	tr	2.7
C _{15:0} 3-OH		1.2							tr			tr		tr		tr			tr	tr	1.1
iso-C _{17:0}	4.1		tr	tr		2.6	tr		1.3			tr	tr	tr	tr	1.1	1.1		tr	tr	
anteiso-C _{17:0}	tr	tr	tr			tr	tr		tr	tr			tr	tr	tr	2.3	tr		tr	tr	
C _{17:1} ω6c	1.2	tr	tr			1.9	6.3		tr				1.0	tr		tr	tr		1.1		tr
C _{17:0}		tr	tr			tr					tr			tr	tr	tr	tr				
iso-C _{16:0} 3-OH	tr	6.1	tr	1.1		tr	tr	1.2	tr	tr		tr	tr	1.1	tr	tr	tr	6.3	11.5	tr	tr
C _{16:0} 3-OH	tr	3.5	2.5	5.1	2.7	tr	tr	tr	1.3	2.4	1.8	3.4	1.7	1.4	1.6	1.7	1.8	7.1	1.3	2.9	5.1
C _{18:1} ω9c		tr	tr			1.5	tr				3.9			tr	tr		1.9		tr	tr	tr
C _{18:0}		3.7	1.3			3.7	tr		tr	1.7	6.8	tr	tr	2.4	1.9	tr	3.9	tr	1.1	1.8	tr
iso-C _{17:0} 3-OH	19.3	12.3	15.4	16.4	12.9	10.6	8.1	16.9	21.8	14.8	8.6	12.0	14.4	25.9	23.9	23.4	16.3	11.4	3.6	16.8	12.7
C _{17:0} 2-OH	tr	1.2	tr	tr		tr	tr	tr	tr	tr		tr	tr	2.9	1.8	2.3	1.3	3.6	2.1	tr	tr
C _{17:0} 3-OH	tr	tr			1.1	tr	tr		tr	tr		tr		1.5	tr	tr	tr		tr	1.0	1.5
Unknown 13.565	1.9	tr	tr	2.8	1.5	tr	tr	8.4	tr		tr	tr	tr	tr	tr	tr		tr	tr		tr
Unknown 14.959																			1.6		
Unknown 16.582	1.9	tr	1.1	1.0		1.0	tr	1.7	1.7	1.9	1.0	1.0	1.1	1.9	2.9	1.9	1.2	tr	tr	1.3	1.0

Table 3. cont.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Summed features*																					
1	5.6					3.4	3.2												tr		
3	6.9	4.0	12.7	16.0	8.3	10.3	10.5	4.7	11.6	10.9	10.7	14.3	9.4	6.8	6.2	5.7	3.2	tr	4.3	7.9	18.1
4	1.8					2.0	tr	1.0													
5	1.4												tr	tr		tr	tr			tr	
8		1.3	tr			1.1					3.1										
9			tr			1.2	tr	5.9													2.3

*Summed features are combinations of fatty acids that cannot be separated by the MIDI system. Summed feature 1 comprises C_{13:0} 3-OH and/or iso-C_{15:1} H; summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1} ω7c; summed feature 4 comprises anteiso-C_{17:1} B and/or iso-C_{17:1} I; summed feature 5 comprises anteiso-C_{18:0} and/or C_{18:2} ω6, 9c; summed feature 8 comprises C_{18:1} ω7c and/or C_{18:1} ω6c; summed feature 9 comprises iso-C_{17:1} ω9c and/or C_{16:0} 10-methyl.

References

- An, D. S., Lee, H. G., Im, W. T., Liu, Q. M. & Lee, S. T. (2007). *Segetibacter koreensis* gen. nov., sp. nov., a novel member of the phylum Bacteroidetes, isolated from the soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* 57, 1828–1833.
- Choi, D. H., Zhang, G. I., Noh, J. H., Kim, W. S. & Cho, B. C. (2009). *Gracilimonas tropica* gen. nov., sp. nov., isolated from a *Synechococcus* culture. *Int J Syst Evol Microbiol* 59, 1167–1172.
- Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* 57, 2259–2261.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fitch, W. M. (1971). Towards defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 20, 406–416.
- Garrity, G. M. & Holt, J. G. (2001). The road map to the *Manual*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 119–166. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Güde, H. (1980). Occurrence of cytophagas in sewage plants. *Appl Environ Microbiol* 39, 756–763.
- Kämpfer, P., Young, C. C., Sridhar, K. R., Arun, A. B., Lai, W. A., Shen, F. T. & Rekha, P. D. (2006). Transfer of [*Flexibacter*] *sancti*, [*Flexibacter*] *filiformis*, [*Flexibacter*] *japonensis* and [*Cytophaga*] *arvensicola* to the genus *Chitinophaga* and description of *Chitinophaga skermanii* sp. nov. *Int J Syst Evol Microbiol* 56, 2223–2228.
- Kämpfer, P., Lodders, N. & Falsen, E. (2011). *Hydrotalea flava* gen. nov. sp. nov., a new species of the phylum Bacteroidetes and allocation of the genera *Chitinophaga*, *Sediminibacterium*, *Lacibacter*, *Flaviumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas* and *Hydrotalea* to the family *Chitinophagaceae* fam. nov. *Int J Syst Evol Microbiol* 61, 518–523.
- Kim, B. Y., Weon, H. Y., Yoo, S. H., Hong, S. B., Kwon, S. W., Stackebrandt, E. & Go, S. J. (2007). *Niabella aurantiaca* gen. nov., sp. nov., isolated from a greenhouse soil in Korea. *Int J Syst Evol Microbiol* 57, 538–541.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16, 111–120.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–147. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lim, J. H., Baek, S.-H. & Lee, S.-T. (2009). *Ferruginibacter alkalilentus* gen. nov., sp. nov. and *Ferruginibacter lapsinensis* sp. nov., novel members of the family 'Chitinophagaceae' in the phylum Bacteroidetes, isolated from freshwater sediment. *Int J Syst Bacteriol* 59, 2394–2399.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 39, 159–167.
- MIDI (1999). Sherlock microbial identification system, operating manual, version 3.0. Newark, DE: MIDI, Inc.
- Qu, J. H. & Yuan, H. L. (2008). *Sediminibacterium salmoneum* gen. nov., sp. nov., a member of the phylum Bacteroidetes isolated from sediment of a eutrophic reservoir. *Int J Syst Evol Microbiol* 58, 2191–2194.
- Qu, J. H., Yuan, H. L., Yang, J. S., Li, H. F. & Chen, N. (2009). *Lacibacter cauensis* gen. nov., sp. nov., a novel member of the phylum

- Bacteroidetes* isolated from sediment of a eutrophic lake. *Int J Syst Evol Microbiol* **59**, 1153–1157.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schenkel, E., Berlaimont, V., Dubois, J., Helson-Cambier, M. & Hanocq, M. (1995). Improved high-performance liquid chromatographic method for the determination of polyamines as their benzoylated derivatives: application to P388 cancer cells. *J Chromatogr B Biomed Appl* **668**, 189–197.
- Sheu, S.-Y., Cho, N.-T., Arun, A. B. & Chen, W.-M. (2010). *Terrimonas aquatica* sp. nov., isolated from a freshwater spring. *Int J Syst Evol Microbiol* **60**, 2705–2709.
- Shiratori, H., Tagami, Y., Morishita, T., Kamihara, Y., Beppu, T. & Ueda, K. (2009). *Filimonas lacunae* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from fresh water. *Int J Syst Evol Microbiol* **59**, 1137–1142.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Woods & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Urios, L., Agogu, H., Lesongeur, F., Stackebrandt, E. & Lebaron, P. (2006). *Balneola vulgaris* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* from the north-western Mediterranean Sea. *Int J Syst Evol Microbiol* **56**, 1883–1887.
- Weon, H. Y., Kim, B. Y., Yoo, S. H., Lee, S. Y., Kwon, S. W., Go, S. J. & Stackebrandt, E. (2006). *Niastella koreensis* gen. nov., sp. nov. and *Niastella yeongjuensis* sp. nov., novel members of the phylum *Bacteroidetes*, isolated from soil cultivated with Korean ginseng. *Int J Syst Evol Microbiol* **56**, 1777–1782.
- Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 241–245. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Greene Publishing and Wiley-Interscience.
- Xie, C. H. & Yokota, A. (2003). Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.
- Xie, C. H. & Yokota, A. (2006). Reclassification of [*Flavobacterium*] *ferrugineum* as *Terrimonas ferruginea* gen. nov., comb. nov., and description of *Terrimonas lutea* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 1117–1121.
- Xu, P., Li, W.-J., Tang, S.-K., Zhang, Y.-Q., Chen, G.-Z., Chen, H.-H., Xu, L.-H. & Jiang, C.-L. (2005). *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family ‘*Oxalobacteraceae*’ isolated from China. *Int J Syst Evol Microbiol* **55**, 1149–1153.
- Yoon, M. H. & Im, W. T. (2007). *Flavisolibacter ginsengiterrae* gen. nov., sp. nov. and *Flavisolibacter ginsengisoli* sp. nov., isolated from ginseng cultivating soil. *Int J Syst Evol Microbiol* **57**, 1834–1839.
- Zhang, K. D., Tang, Y. L., Zhang, L., Dai, J., Wang, Y., Luo, X. S., Liu, M., Luo, G. C. & Fang, C. X. (2009). *Parasegetibacter luojiensis* gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from a forest soil. *Int J Syst Evol Microbiol* **59**, 3058–3062.
- Zhang, N. N., Qu, J.-H., Yuan, H.-L., Sun, Y. M. & Yang, J.-S. (2010). *Flaviumibacter petaseus* gen. nov., sp. nov., isolated from soil of a subtropical rainforest. *Int J Syst Evol Microbiol* **60**, 1609–1612.