Correspondence

sqli@ms.iswc.ac.cn

Shiqing Li.

Nafulsella turpanensis gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from soil

Lei Zhang,^{1,2} Xihui Shen,¹ Yingbao Liu¹ and Shiqing Li^{1,2,3}

¹State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, PR China

²State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Northwest A&F University, Yangling, Shaanxi 712100, PR China

³ College of Resource and Environment, Northwest A&F University, Yangling, Shaanxi 712100, PR China

A Gram-staining-negative, rod-shaped, gliding and pale-pink-pigmented bacterium, designated strain ZLM-10^T, was isolated from a soil sample collected from an arid area in Xinjiang province, China, and characterized in a taxonomic study using a polyphasic approach. The novel strain grew optimally at 30-37 °C and in the presence of 2 % (w/v) sea salts. The only respiratory quinone detected was MK-7 and the major cellular fatty acids were summed feature 3 (iso-C15:0 2-OH and/or $C_{16\cdot1}\omega7c$), iso- $C_{15\cdot0}$ and iso- $C_{17\cdot0}$ 3-OH. The polar lipids consisted of diphosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified aminophospholipids. The DNA G+C content was 45.4 mol%. Flexirubin-type pigments were not produced. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ZLM-10^T was a member of the phylum *Bacteroidetes* and appeared most closely related to Cesiribacter roseus 311^T (90.2 % sequence similarity), Marivirga sericea LMG 13021^T (89.2 %), Cesiribacter and amanensis AMV16^T (89.1%) and Marivirga tractuosa DSM 4126^T (89.1%). On the basis of phenotypic and genotypic data and phylogenetic inference, strain ZLM-10^T should be classified as a novel species of a new genus in the family Flammeovirgaceae, for which the name Nafulsella turpanensis gen. nov., sp. nov. is proposed. The type strain of the type species is ZLM-10^T (=CCTCC AB 208222^T=KCTC 23983^T).

The family Flammeovirgaceae belongs to the phylum Bacteroidetes and was first proposed by Garrity & Holt (2001) in the second edition of Bergey's Manual of Systematic Bacteriology. Recently, the description of the family Flammeovirgaceae was formally established by Yoon et al. (2011a). At the time of writing, the family Flammeovirgaceae incorporates the genera Aureibacter, Cesiribacter, Fabibacter, Flammeovirga, Flexithrix, Fulvivirga, Limibacter, Marivirga, Perexilibacter, Persicobacter, 'Porifericola', Rapidithrix, Reichenbachiella, Roseivirga, Sediminitomix and 'Tunicatimonas' (Ludwig et al., 2010; Nedashkovskaya et al., 2010; Srinivas et al., 2011; Yoon et al., 2011a, b, 2012). In the present study, we report on the taxonomic characterization of a pale-pink-pigmented bacterium, designated strain ZLM-10^T, that was isolated from a soil sample collected from an arid area in Xinjiang province, China. Based on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ZLM- 10^{T} is JN899241.

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

phenotypic and genotypic data and phylogenetic analysis, it is proposed that the new isolate represents a novel species in a new genus of the family *Flammeovirgaceae*.

For strain isolation, the soil sample was thoroughly suspended in sterile water and serial dilutions were spread on marine agar 2216 (MA; Difco). Isolation was achieved after incubation at 30 °C for 1 week. Strain ZLM- 10^{T} was routinely cultivated on the same medium at 30 °C and preserved at -80 °C in marine broth 2216 (MB; Difco) supplemented with 20% (v/v) glycerol.

Genomic DNA of strain ZLM- 10^{T} was prepared according to the procedure of Wilson (1987) with the modification by Cleenwerck *et al.* (2002). Genomic DNA G+C content was determined by HPLC (UltiMate 3000; Dionex) according to the method of Mesbah *et al.* (1989). The 16S rRNA gene sequence of strain ZLM- 10^{T} was amplified by PCR with the bacterial universal primers 27f and 1527r (Lane, 1991), and the PCR products were sequenced commercially, by Invitrogen Biotechnology. Identification

led from www.microbiologyresearch.org by

of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). Phylogenetic analysis was performed using version 4.1 of the MEGA package (Tamura *et al.*, 2007), after multiple alignment of the data within CLUSTAL_X (Thompson *et al.*, 1997). Phylogenetic distances were calculated by the neighbour-joining method (Saitou & Nei, 1987), using the Kimura two-parameter model (Kimura, 1980), and by 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 (1441 nt) of strain ZLM-10^T was determined. Comparative 16S rRNA gene sequence analysis revealed that strain ZLM-10^T was affiliated with the family *Flammeovirgaceae* within the phylum *Bacteroidetes*, and exhibited the highest sequence similarities to *Cesiribacter roseus* 311^T (90.2 %), *Marivirga sericea* LMG 13021^T (89.2 %), *Cesiribacter andamanensis* AMV16^T (89.1 %) and *Marivirga tractuosa* DSM 4126^T (89.1 %). All other species of the family *Flammeovirgaceae* with validly published names were more distantly related, showing 16S rRNA gene sequence similarities of <88.9 %. In the phylogenetic tree based on the neighbour-joining algorithm, strain ZLM-10^T was clustered with members of the genera *Cesiribacter* and *Marivirga* but was clearly separated from these two genera (Fig. 1). The overall topology of

the maximum-parsimony tree was essentially the same as that of the neighbour-joining tree (data not shown). The genomic DNA G+C content of strain ZLM- 10^{T} was 45.4 mol%, which differs from the DNA G+C contents of its phylogenetically closest relatives (Table 1). These results suggest that strain ZLM- 10^{T} represents a novel genus within the family *Flammeovirgaceae*.

Growth was evaluated at 30 °C on several standard bacteriological media: marine agar 2216 (MA; Difco), R2A agar (Difco), trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco) and MacConkey agar (Difco). Cell morphology was examined by transmission electron microscopy (H-8100; Hitachi) using cells from the exponential growth phase. The cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gram staining of cells was carried out according to the classical Gram procedure described by Doetsch (1981). Gliding motility was determined as described by Bowman (2000). The presence of flexirubintype pigments was investigated using the bathochromatic shift test, with 20 % (w/v) KOH (Bernardet et al., 2002; McCammon & Bowman, 2000). The temperature range and optimum for growth were assessed at 4, 7, 10, 15, 20, 25, 30, 37, 40, 42, 45 and 50 °C on MA for up to 1 week. The pH range and optimum for growth were tested in MB medium at pH 4.0-11.0, by using 0.1 M citric acid/0.1 M sodium citrate (for pH 4.0-5.0), 0.1 M KH₂PO₄/0.1 M NaOH (for pH 6.0-8.0), 0.1 M NaHCO₃/0.1 M Na₂CO₃ (for pH 9.0-10.0) or 0.05 M Na₂HPO₄/0.1 M NaOH (for

0.02 100 $Rest 72 Rost61 100 F99 100 R100 R61 100 R99 100 R56 84 79 100 R56 84 79 100 R56 95 100 R$	eivirga ehrenbergii KMM 6017 ^T (AY608410) iseivirga echinicomitans KMM 6058 ^T (AY753206) ivirga spongicola UST030701-084 ^T (DQ080996) abibacter halotolerans UST030701-097 ^T (DQ080995) - Marinoscillum pacificum MRN461 ^T (DQ660388) Marinoscillum furvescens NBRC 15994 ^T (AB078079) ulvivirga kasyanovii KMM 6220 ^T (DQ836305) - Reichenbachiella agariperforans KMM 3525 ^T (AB058919) - Reichenbachiella agariperforans KMM 3525 ^T (AB058919) - Reichenbachiella faecimaris PCP11 ^T (GU143096) - 'Porifericola rhodea' N5EA6-3A2B (AB602436) - 'Porifericola rhodea' N5EA6-3A2B (AB602437) Marivirga sericea LMG 13021 ^T (AB078081) larivirga sericea LMG 13021 ^T (AB078081) larivirga tractuosa NBRC 15989 ^T (AB078072) - Nafulsella turpanensis ZLM-10 ^T (JN899241) - Cesiribacter roseus 311 ^T (HM775387) - Cesiribacter aerophilus 6424S-25 ^T (GQ421850) - Adhaeribacter aerophilus 6424S-25 ^T (GQ421850) - Adhaeribacter aerophilus 6424S-25 ^T (GQ421846) - Adhaeribacter aquaticus MBRG1.5 ^T (AJ626894) reibacter tunicatorum A5Q-118 ^T (AB572584) - Persicobacter psychrovividus Asr22-19 ^T (AB260932) - Flexithrix dorotheae NBRC 15987 ^T (AB265192) - Flexithrix dorotheae NBRC 15987 ^T (AB265192) - Sediminitomix flava NBRC 101625 ^T (AB255370) - Limibacter armeniacum YM11-185 ^T (AB359907) - Perexilibacter aurantiacus MBIC06993 ^T (AB276355) - Flammeovirga aprica NBRC 15984 ^T (AB078078)	Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships between strain ZLM- 10^{T} and related taxa. Bootstrap values >50% (based on 1000 replications) are shown at branching points. <i>Thermonema lapsum</i> ATCC 43542 ^T was used as the outgroup. Bar, 0.02
	¹⁰⁰ — Flammeovirga arenaria NBRC 15982 [™] (AB078078) — Thermonema lapsum ATCC 43542 [™] (L11703)	43542 ¹ was used as the outgroup. Bar, 0.02 substitutions per nucleotide position.

Strains: 1, ZLM-10^T; 2, *C. roseus* 311^T; 3, *M. tractuosa* DSM 4126^T. All data were from this study except for the genomic DNA G+C contents of strains 2 and 3, which were taken from Liu *et al.* (2012) and Nedashkovskaya *et al.* (2010), respectively. All three strains are positive for growth on MA, hydrolysis of gelatin, and catalase, alkaline phosphatase, esterase (C4), esterase lipase(C8), leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase and α -glucosidase activities. All strains are negative for growth on MacConkey agar, H₂S and indole production, citrate utilization, arginine dihydrolase, urease, ornithine decarboxylase, lysine decarboxylase, β -glucuronidase, α -mannosidase and α -fucosidase activities, and acid production from L-arabinose, D-mannitol, D-galactose, D-mannose, L-rhamnose, glycerol, D-xylose, D-arabinose and lactose. +, Positive; -, negative; w, weakly positive.

Characteristic	1	2	3			
Motility	Gliding	+	Gliding			
Oxidase	+	_	+			
Growth on:						
R2A agar	_	+	W			
$0.1 \times TSA$	_	+	_			
TSA	_	_	+			
NA	_	_	W			
Temperature range for growth (°C)	10-45	4–37	10-40			
Salinity range (%, w/v)	0.5-6*	0–3	0.5–6			
Production of flexirubin-type pigments	_	+	+†			
Hydrolysis of:						
Aesculin	_	+	+			
Starch	+	+	_			
Nitrate reduction	_	+	_			
Voges–Proskauer reaction	+	_	_			
Tryptophan deaminase	_	+	_			
Acid production from:						
D-Glucose	+	_	_			
D-Fructose	+	_	_			
D-Ribose	w	W	_			
Maltose	+	W	_			
Sucrose	W	_	_			
Trehalose	_	W	_			
Enzyme activities (API ZYM):						
Lipase (C14)	W	-	-			
Cystine arylamidase	+	—	+			
Trypsin	+	-	_			
Acid phosphatase	+	-	+			
α-Galactosidase	—	+	_			
α-Chymotrypsin	-	_	+			
β -Galactosidase	_	-	+			
β -Glucosidase	_	_	+			
<i>N</i> -Acetyl- β -glucosaminidase	-	+	-			
DNA G+C content (mol%)	45.4	47.1	36.1			

*Strain ZLM-10^T did not grow with NaCl as the only salt; the values given refer to sea salts.

†Negative result reported by Nedashkovskaya et al. (2010).

pH 11.0) as buffer. Growth in the absence of NaCl and in the presence of 0.5–10.0 % (w/v) NaCl (at 0.5 % intervals) was investigated in peptone-yeast extract (PY) medium that contained (1^{-1}) 2.5 g Bacto peptone and 0.5 g yeast extract. Growth was also determined in PY medium containing sea salts (Sigma) at 0-10.0 % (w/v) (in increments of 0.5%). Growth under anaerobic conditions was determined after 2 weeks of incubation on MA in an anaerobic jar containing an AnaeroGen anaerobic system envelope (Oxoid). Oxidase activity was determined by using 1% (w/v) tetramethyl-*p*-phenylenediamine. Catalase activity was detected by assessing the production of bubbles after the addition of a drop of 3 % (v/v) H₂O₂. Hydrolysis of casein and starch was assessed according to the methods of Smibert & Krieg (1994). Hydrolysis of carboxymethycellulose (0.1%, w/v), chitin from crab shells (1%, w/v) and tyrosine (0.5%, w/v) was also assessed. Acid production from carbohydrates was determined as described by Leifson (1963). Activities of constitutive enzymes and other physiological properties were determined by using API 20E, API 20NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions, except that all of the suspension media used for strain ZLM-10^T were supplemented with 2% (w/v) sea salts. Antimicrobial susceptibility testing was performed by the agar-diffusion method using antibiotic-impregnated discs, as described by Buczolits et al. (2002).

At 30 °C, strain ZLM-10^T grew well on MA but not on R2A agar, TSA, NA or MacConkey agar. Cells of strain ZLM-10^T were strictly aerobic, Gram-staining-negative, gliding and rod-shaped (Fig. 2). Strain ZLM-10^T did not grow in PY medium containing NaCl (at $\leq 10\%$, w/v) as the only salt but did grow in PY medium supplemented with 0.5–6.0% (w/v) sea salts (optimum, 2.0%). Flexirubin-type pigments were not produced. Other phenotypic features of strain ZLM-10^T are summarized in the species description. Strain ZLM-10^T differed from its closest phylogenetic relative, *Cesiribacter roseus* 311^T, in several phenotypic characteristics, including its mode of motility, temperature range for growth, tolerance of salt, hydrolysis of aesculin, nitrate reduction, Voges–Proskauer reaction, acid production

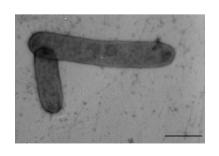


Fig. 2. Transmission electron micrograph showing the general morphology of negatively stained cells of strain ZLM-10^T after growth for 36 h at 30 °C on MA. Bar, 800 nm.

Table 2. Distinguishing features of strain ZLM-10^T and genera within the family *Flammeovirgaceae*

Taxa: 1, strain ZLM-10^T (data from this study); 2, *Cesiribacter* (Srinivas *et al.*, 2011; Liu *et al.*, 2012); 3, *Marivirga* (Nedashkovskaya *et al.*, 2010); 4, *Aureibacter* (Yoon *et al.*, 2011a); 5, *Fabibacter* (Lau *et al.*, 2006); 6, *Flammeovirga* (Takahashi *et al.*, 2006; Hosoya & Yokota, 2007a; Xu *et al.*, 2012); 7, *Flexithrix* (Lewin, 1970; Hosoya & Yokota, 2007b); 8, *Fulvivirga* (Nedashkovskaya *et al.*, 2007; Nupur *et al.*, 2012); 9, *Limibacter* (Yoon *et al.*, 2007); 11, *Persicobacter* (Muramatsu *et al.*, 2010); 12, '*Porifericola*' (Yoon *et al.*, 2011b); 13, *Rapidithrix* (Srisukchayakul *et al.*, 2007); 14, *Reichenbachiella* (Nedashkovskaya *et al.*, 2003, 2005a; Cha *et al.*, 2011); 15, *Roseivirga* (Nedashkovskaya *et al.*, 2005b, c, 2008; Lau *et al.*, 2006); 16, *Sediminitomix* (Khan *et al.*, 2007); 17, '*Tunicatimonas*' (Yoon *et al.*, 2012). +, Positive; –, negative; w, weakly positive; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Gliding motility	+	_	+	_	+	+	+	v	+	+	+	_	+	+	v	_	_
Nitrate reduction	_	+	_	_	-	+	_	v	_	—	+	_	ND	_	v	+	_
Oxidase	+	V	+	+	+	V	+	+	+	+	_	-	+	+	+	+	_
Catalase	+	+	+	+	+	V	+	+	+	+	+	+	_	+	+	+	+
Hydrolysis of:																	
Gelatin	+	V	+	+	_	V	_	V	+	+	+	+	+	V	+	+	_
Starch	+	V	_	+	W	V	_	V	_	_	W	-	+	+	_	+	_
Casein	+	V	V	ND	-	V	-	V	ND	ND	ND	ND	ND	V	_	_	_
Voges-Proskauer reaction	+	_	-	-	+	+	-	ND	-	_	ND	+	ND	ND	V	ND	_
Growth at 45 $^\circ C$	+	-	-	-	-	-	-	_	_	_	V	-	ND	_	_	_	_
Enzyme activities (API ZYM):																	
Acid phosphatase	+	-/ND	+	+	+	+	+	ND	+	+	ND	+	+	+	+	+	+
Alkaline phosphatase	+	+/ND	+	+	+	+	+	ND	+	+	ND	+	+	+	+	+	+
Leucine arylamidase	+	+/ND	+	+	+	+	+	ND	+	+	ND	+	+	+	+	+	+
Valine arylamidase	+	+/ND	+	+	+	+	+	ND	+	+	ND	+	+	+	+	+	+
Esterase (C4)	+	+/ND	+	+	+	w	+	ND	_	_	ND	+	+	V	+	ND	+
α-Fucosidase	_	-/ND	_	_	_	_	+	ND	_	_	ND	_	+	_	_	ND	_
DNA $G + C$ content	45.4	47.1	36.1	36.2	42.5	31–36	35.6	55.1	27.9	43.0	42.0	43.0	40	39.6	40.2	38	52.6
(mol%)		-50.9	-37.1					-59.9			-43.8		-43	-44.5	-43.7		

from carbohydrates and some enzyme activities (Table 1). Strain ZLM- 10^{T} could also be differentiated phenotypically from *Marivirga tractuosa* DSM 4126^{T} , the type species of the genus *Marivirga* (Table 1). The phenotypic features differentiating strain ZLM- 10^{T} from related members of the family *Flammeovirgaceae* are shown in Table 2.

The respiratory quinones of strain ZLM-10^T were extracted and identified by HPLC, as described by Xie & Yokota (2003). Polar lipids of strain ZLM-10^T were extracted and analysed as described by Tindall (1990). For this, 6.75 ml of chloroform/methanol/saline [0.3 % (w/v) NaCl in water] (1:2:0.8, by vol.) was added to 100 mg freeze-dried cells. The preparation was stirred overnight before the cell debris was pelleted by centrifugation. Polar lipids were recovered into the chloroform phase, by adjusting the chloroform/ methanol/saline mixture to a ratio of 1:1:0.9 (by vol.), and then dried under nitrogen. The dried polar lipids were resuspended in chloroform/methanol (2:1, v/v) and separated by two-dimensional TLC. The total lipids and specific functional groups were detected using 5% (w/v) ethanolic molybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (a-glycols), Dragendorff's reagent (quaternary nitrogen) and anisaldehyde-sulfuric acid (glycolipids). For cellular fatty acid analysis, samples of cell

biomass of strain ZLM-10^T, *Cesiribacter roseus* 311^T and *Marivirga tractuosa* DSM 4126^T were harvested at lateexponential growth phase from MA plates incubated at 30 °C. The physiological age of the three cultures was standardized according to the protocol given by MIDI (http:// www.microbialid.com/PDF/TechNote_101.pdf). Fatty acids were saponified, methylated and extracted using the standard protocol of version 6.0 of the Sherlock Microbial Identification System (MIDI), analysed by GC (6890N; Hewlett Packard) and identified using the TSBA6 database of the Microbial Identification System (Sasser, 1990).

The only isoprenoid quinone detected in strain ZLM- 10^{T} was menaquinone 7 (MK-7). The polar lipid profile (Fig. S1, available in IJSEM Online) consisted mainly of phosphatidylethanolamine but diphosphatidylglycerol and two unidentified aminophospholipids occurred at moderate levels and trace amounts of an unidentified aminolipid (AL1) were also detected. The major fatty acids of strain ZLM- 10^{T} were summed feature 3 ($C_{16:1}\omega 7c$ and/ or $C_{16:1}\omega 6c$; 26.4 %), iso- $C_{15:0}$ (20.3 %) and iso- $C_{17:0}$ 3-OH (12.0 %). In addition, $C_{16:1}\omega 5c$ (5.7 %), anteiso- $C_{15:0}$ (5.5 %), summed feature 9 (iso- $C_{17:1}\omega 9c$ and/or 10-methyl $C_{16:0}$; 4.4 %) and iso- $C_{17:0}$ (4.0 %) were detected in moderate amounts. As shown in Table 3, the fatty acid profile of strain ZLM- 10^{T} was distinct from that of

Table 3. Cellular fatty acid contents (%) of strain ZLM-10^T, Cesiribacter roseus 311^T and Marivirga tractuosa DSM 4126^T

Strains: 1, ZLM-10^T; 2, *C. roseus* 311^T; 3, *M. tractuosa* DSM 4126^T. All data from this study; the three strains were cultured on MA at 30 °C and cells at late-exponential growth phase were used. Fatty acids amounting to <1% of the total fatty acids in all three strains are not listed. TR, Trace (<1%); -, not detected.

Fatty acid	1	2	3
iso-C _{13:0}	TR	TR	1.0
iso-C _{14:0}	TR	-	2.8
iso-C _{15:1} G	TR	2.9	_
iso-C _{15:0}	20.3	21.6	31.0
anteiso-C _{15:0}	5.5	TR	TR
iso-C _{16:1} H	TR	-	4.8
iso-C _{16:0}	2.1	TR	12.8
$C_{16:1}\omega 5c$	5.7	32.1	-
C _{16:0}	3.5	1.3	1.1
iso-C _{15:0} 3-OH	3.0	2.9	4.5
iso-C _{17:0}	4.0	2.9	TR
anteiso-C _{17:0}	1.2	TR	-
$C_{17:1}\omega 6c$	TR	TR	2.2
iso-C _{16:0} 3-OH	TR	TR	4.0
С _{16:0} 3-ОН	TR	TR	2.0
iso-C _{17:0} 3-OH	12.0	14.3	9.7
C _{17:0} 2-OH	1.0	TR	_
Summed features*			
3	26.4	1.0	6.0
4	2.1	14.6	-
8	1.6	-	-
9	4.4	_	12.4

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 4 contained anteiso- $C_{17:1}$ B and/or iso- $C_{17:1}$ I; summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9 contained iso- $C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

Cesiribacter roseus 311^{T} , the novel strain having summed feature 9 as well as significantly smaller amounts of $C_{16:1}\omega5c$ and summed feature 4 and significantly larger amounts of summed feature 3 and anteiso- $C_{15:0}$. Fatty acid profiles also allowed strain ZLM- 10^{T} to be differentiated from *Marivirga tractuosa* DSM 4126^T, the novel strain having $C_{16:1}\omega5c$ as well as a significantly smaller amount of summed feature 9 and significantly larger amounts of summed feature 3 and anteiso- $C_{15:0}$.

Based on the phenotypic and genotypic data and the relatively low levels of 16S rRNA gene sequence similarity between strain ZLM-10^T and members of related genera of the family *Flammeovirgaceae*, strain ZLM-10^T represents a novel species of a new genus in the family *Flammeovirgaceae*, for which the name *Nafulsella turpanensis* gen. nov., sp. nov. is proposed.

Description of Nafulsella gen. nov.

Nafulsella (Na.ful.sel'la. N.L. fem. dim. n. *Nafulsella* arbitrary name derived from the acronym, NAFULS, used for the Northwest A&F University College of Life Sciences, where the type species was identified).

Cells are rod-shaped, Gram-stain-negative and strictly aerobic. Motile by gliding. Catalase- and oxidase-positive. Flexirubin-type pigments are not detected. Negative for nitrate and nitrite reduction. The major respiratory menaquinone is MK-7. Predominant cellular fatty acids are summed feature 3 (iso- $C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), iso- $C_{15:0}$ and iso- $C_{17:0}$ 3-OH. The major polar lipid is phosphatidylethanolamine. The type species of the genus is *Nafulsella turpanensis*.

Description of Nafulsella turpanensis sp. nov.

Nafulsella turpanensis (tur.pan.en'sis. N.L. fem. adj. *turpanensis* of or pertaining to Turpan, the city in Xinjiang province, north-western China where the type strain was isolated).

Cells are strictly aerobic, Gram-stain-negative rods that measure approximately 0.5-0.6 µm in width and 1.4-3.2 µm in length and are motile by gliding. Colonies grown on MA for 3 days are pale pink, smooth, circular, flat and 1-2 mm in diameter, with clear margins. Growth occurs at 10-45 °C (optimum 30-37 °C), at pH 6-9 (optimum, pH 7) and in the presence of 0.5-6% (w/v) sea salts (optimum, 2%). Unable to grow with NaCl as the sole salt. Hydrolyses gelatin, starch and casein but not aesculin, carboxymethylcellulose, chitin or tyrosine. Positive for acetoin production (Voges-Proskauer reaction). Negative for H₂S production, citrate utilization, indole production and arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities (API 20E). Negative for the assimilation of D-glucose, Larabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid (API 20NE). Acid is produced from D-glucose, maltose, sucrose (weakly), D-fructose and D-ribose (weakly) but not from D-mannitol, glycerol, D-arabinose, L-arabinose, Dxylose, D-galactose, D-mannose, L-rhamnose, lactose or trehalose. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14) (weakly), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BIphosphohydrolase and α -glucosidase activities, but negative for α -chymotrypsin, α -galactosidase, β -galactosidase, β glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase activities. Susceptible to (µg per disc unless indicated otherwise) ampicillin (10), penicillin G (10 IU), carbenicillin (100), chloramphenicol (30), doxycycline (30), erythromycin (15), lincomycin (15), tetracycline (30) and vancomycin (30), but resistant to gentamicin (10), kanamycin (30), neomycin (30) and streptomycin (10). The only respiratory quinone is MK-7. The polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, an unidentified aminolipid and two unidentified aminophospholipids. The major fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), iso- $C_{15:0}$ and iso- $C_{17:0}$ 3-OH. The detailed fatty acid composition is given in Table 3.

The type strain, ZLM- 10^{T} (=CCTCC AB 208222^T=KCTC 23983^T), was isolated from a soil sample collected from an arid area in Turpan city, Xinjiang province, north-western China. The genomic DNA G+C content of the type strain is 45.4 mol%.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (no. 31100001), the China Postdoctoral Science Foundation (no. 20100481373) and Northwest A&F University (no. Z111020910).

References

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of *Flavobacterium* and *Cytophaga*-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* 52, 1049–1070.

Bowman, J. P. (2000). Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Cellulophaga uliginosa* comb. nov. *Int J Syst Evol Microbiol* **50**, 1861–1868.

Buczolits, S., Denner, E. B. M., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* 52, 445–456.

Cha, I. T., Oh, Y. S., Park, S. J., Park, B. J., Lee, J. K., Lim, C. S., Park, A. R., Yoo, J. S., Lee, D. H. & other authors (2011). *Reichenbachiella faecimaris* sp. nov., isolated from a tidal flat of Yellow Sea, Korea, and emended descriptions of the genus *Reichenbachiella* and of *Reichenbachiella agariperforans*. *Int J Syst Evol Microbiol* **61**, 1994–1999.

Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* 52, 1551–1558.

Doetsch, R. N. (1981). Determinative methods of light microscopy. In *Manual of Methods for General Bacteriology*, pp. 21–33. Edited by P. Gerdhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington, DC: American Society for Microbiology.

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.

Hosoya, S. & Yokota, A. (2007a). Flammeovirga kamogawensis sp. nov., isolated from coastal seawater in Japan. Int J Syst Evol Microbiol 57, 1327–1330.

Hosoya, S. & Yokota, A. (2007b). Reclassification of *Flexibacter* aggregans (Lewin 1969) Leadbetter 1974 as a later heterotypic synonym of *Flexithrix dorotheae* Lewin 1970. *Int J Syst Evol Microbiol* 57, 1086–1088.

Khan, S. T., Nakagawa, Y. & Harayama, S. (2007). Sediminitomix flava gen. nov., sp. nov., of the phylum *Bacteroidetes*, isolated from marine sediment. *Int J Syst Evol Microbiol* 57, 1689–1693.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62, 716–721.

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. 125–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lau, S. C., Tsoi, M. M., Li, X., Plakhotnikova, I., Dobretsov, S., Wu, M., Wong, P. K., Pawlik, J. R. & Qian, P. Y. (2006). Description of *Fabibacter halotolerans* gen. nov., sp. nov. and *Roseivirga spongicola* sp. nov., and reclassification of [*Marinicola*] *seohaensis* as *Roseivirga seohaensis* comb. nov. Int J Syst Evol Microbiol 56, 1059–1065.

Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 85, 1183–1184.

Lewin, R. A. (1970). *Flexithrix dorotheae* gen. et sp. nov. (Flexibacterales); and suggestions for reclassifying sheathed bacteria. *Can J Microbiol* 16, 511–515.

Liu, M., Qi, H., Luo, X., Dai, J., Peng, F. & Fang, C. (2012). *Cesiribacter roseus* sp. nov., a pink-pigmented bacterium isolated from desert sand. *Int J Syst Evol Microbiol* 62, 96–99.

Ludwig, W., Euzéby, J. & Whitman, W. B. (2010). Taxonomic outlines of the phyla Bacteroidetes, Spirochaetes, Tenericutes (Mollicutes), Acidobacteria, Fibrobacteres, Fusobacteria, Dictyoglomi, Gemmatimonadetes, Lentisphaerae, Verrucomicrobia, Chlamydiae, and Planctomycetes. In Bergey's Manual of Systematic Bacteriology, 2nd edn, vol. 4, pp. 21–24. Edited by N. R. Krieg, J. T. Staley, D. R. Brown, B. P. Hedlund, B. J. Paster, N. L. Ward, W. Ludwig & W. B. Whitman. New York: Springer.

McCammon, S. A. & Bowman, J. P. (2000). Taxonomy of Antarctic Flavobacterium species: description of *Flavobacterium* gillisiae sp. nov., *Flavobacterium tegetincola* sp. nov., and *Flavobacterium xanthum* sp. nov., nom. rev. and reclassification of [*Flavobacterium*] salegens as Salegentibacter salegens gen. nov., comb. nov. Int J Syst Evol Microbiol **50**, 1055–1063.

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.

Muramatsu, Y., Takahashi, M., Kaneyasu, M., Iino, T., Suzuki, K. & Nakagawa, Y. (2010). *Persicobacter psychrovividus* sp. nov., isolated from shellfish, and emended descriptions of the genus *Persicobacter* and *Persicobacter diffluens*. Int J Syst Evol Microbiol **60**, 1735–1739.

Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga–Flavobacterium–Bacteroides. Int J Syst Evol Microbiol* 53, 81–85.

Nedashkovskaya, O. I., Kim, S. B., Suzuki, M., Shevchenko, L. S., Lee, M. S., Lee, K. H., Park, M. S., Frolova, G. M., Oh, H. W. & other authors (2005a). *Pontibacter actiniarum* gen. nov., sp. nov., a novel member of the phylum '*Bacteroidetes*', and proposal of *Reichenbachiella* gen. nov. as a replacement for the illegitimate prokaryotic generic name

International Journal of Systematic and Evolutionary Microbiology 63

Reichenbachia Nedashkovskaya et al. 2003. Int J Syst Evol Microbiol 55, 2583–2588.

Nedashkovskaya, O. I., Kim, S. B., Lee, D. H., Lysenko, A. M., Shevchenko, L. S., Frolova, G. M., Mikhailov, V. V., Lee, K. H. & Bae, K. S. (2005b). *Roseivirga ehrenbergii* gen. nov., sp. nov., a novel marine bacterium of the phylum '*Bacteroidetes*', isolated from the green alga *Ulva fenestrata*. Int J Syst Evol Microbiol 55, 231–234.

Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Park, M. S., Mikhailov, V. V., Bae, K. S. & Park, H. Y. (2005c). *Roseivirga echinicomitans* sp. nov., a novel marine bacterium isolated from the sea urchin *Strongylocentrotus intermedius*, and emended description of the genus *Roseivirga*. *Int J Syst Evol Microbiol* 55, 1797–1800.

Nedashkovskaya, O. I., Kim, S. B., Shin, D. S., Beleneva, I. A. & Mikhailov, V. V. (2007). *Fulvivirga kasyanovii* gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from seawater in a mussel farm. *Int J Syst Evol Microbiol* 57, 1046–1049.

Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Kalinovskaya, N. I. & Mikhailov, V. V. (2008). Reclassification of *Roseivirga seohaensis* (Yoon *et al.* 2005) Lau *et al.* 2006 as a later synonym of *Roseivirga ehrenbergii* Nedashkovskaya *et al.* 2005 and emendation of the species description. *Int J Syst Evol Microbiol* 58, 1194–1197.

Nedashkovskaya, O. I., Vancanneyt, M., Kim, S. B. & Bae, K. S. (2010). Reclassification of *Flexibacter tractuosus* (Lewin 1969) Leadbetter 1974 and '*Microscilla sericea*' Lewin 1969 in the genus *Marivirga* gen. nov. as *Marivirga tractuosa* comb. nov. and *Marivirga sericea* nom. rev., comb. nov. *Int J Syst Evol Microbiol* **60**, 1858–1863.

Nupur, Sharma, S., Kumar Singh, P., Suresh, K. & Anil Kumar, P. (2012). Fulvivirga imtechensis sp. nov., a member of the phylum Bacteroidetes. Int J Syst Evol Microbiol 62, 2213–2217. doi

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. Newark, DE: MIDI Inc.

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.

Srinivas, T. N. R., Anil Kumar, P., Madhu, S., Sunil, B., Sharma, T. V. R. S. & Shivaji, S. (2011). *Cesiribacter andamanensis* gen. nov., sp. nov., isolated from a soil sample from a mud volcano. *Int J Syst Evol Microbiol* **61**, 1521–1527.

Srisukchayakul, P., Suwanachart, C., Sangnoi, Y., Kanjana-Opas, A., Hosoya, S., Yokota, A. & Arunpairojana, V. (2007). *Rapidithrix thailandica* gen. nov., sp. nov., a marine gliding bacterium isolated from samples collected from the Andaman sea, along the southern coastline of Thailand. *Int J Syst Evol Microbiol* **57**, 2275–2279. Takahashi, M., Suzuki, K. & Nakagawa, Y. (2006). Emendation of the genus *Flammeovirga* and *Flammeovirga aprica* with the proposal of *Flammeovirga arenaria* nom. rev., comb. nov. and *Flammeovirga yaeyamensis* sp. nov. Int J Syst Evol Microbiol 56, 2095–2100.

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.

Tindall, B. J. (1990). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 13, 128–130.

Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. 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 & 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.

Xu, H., Fu, Y., Yang, N., Ding, Z., Lai, O. & Zeng, R. (2012). *Flammeovirga pacifica* sp. nov., isolated from deep-sea sediment. *Int J Syst Evol Microbiol* 62, 937–941.

Yoon, J., Ishikawa, S., Kasai, H. & Yokota, A. (2007). *Perexilibacter aurantiacus* gen. nov., sp. nov., a novel member of the family '*Flammeovirgaceae*' isolated from sediment. *Int J Syst Evol Microbiol* 57, 964–968.

Yoon, J., Matsuo, Y., Kasai, H. & Yokota, A. (2008). Limibacter armeniacum gen. nov., sp. nov., a novel representative of the family 'Flammeovirgaceae' isolated from marine sediment. Int J Syst Evol Microbiol 58, 982–986.

Yoon, J., Adachi, K., Park, S., Kasai, H. & Yokota, A. (2011a). *Aureibacter tunicatorum* gen. nov., sp. nov., a marine bacterium isolated from a coral reef sea squirt, and description of *Flammeovirgaceae* fam. nov. *Int J Syst Evol Microbiol* **61**, 2342–2347.

Yoon, J., Oku, N., Park, S., Kasai, H. & Yokota, A. (2011b). *Porifericola rhodea* gen. nov., sp. nov., a new member of the phylum *Bacteroidetes* isolated by the bait-streaked agar technique. *Antonie van Leeuwenhoek* 100, 145–153.

Yoon, J., Oku, N., Park, S., Katsuta, A. & Kasai, H. (2012). *Tunicatimonas pelagia* gen. nov., sp. nov., a novel representative of the family *Flammeovirgaceae* isolated from a sea anemone by the differential growth screening method. *Antonie van Leeuwenhoek* 101, 133–140.