Sediminibacterium salmoneum gen. nov., sp. nov., a member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic reservoir

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Strain NJ-44^T, isolated from sediment of the eutrophic Guanting Reservoir in Beijing (China), was subjected to a taxonomic study using a polyphasic approach. The strain was aerobic, with salmon-pink-pigmented colonies on R2A agar. Cells were single, Gram-negative rods, motile by gliding. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain NJ-44^T belonged to the phylum *Bacteroidetes*, with *Terrimonas ferruginea* ATCC 13524^T (90.8 % similarity), *Terrimonas lutea* DY^T (90.5 %) and *Niabella aurantiaca* R2A15-11^T (89.1 %) as its closest relatives. Strain NJ-44^T was clearly differentiated from members of the genera *Terrimonas* and *Niabella* in its DNA G + C content (40.6 mol%) and its major fatty acids, iso-C_{15:1} G, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} 3-OH, iso-C_{17:0} 3-OH, anteiso-C_{15:1} A and iso-C_{15:0} 3-OH. It is proposed that strain NJ-44^T represents a novel genus and species, named *Sediminibacterium salmoneum* gen. nov., sp. nov. The type strain of *Sediminibacterium salmoneum* is strain NJ-44^T (=CGMCC 1.6845^T =NBRC 103935^T).

During the study of the bacterial community in the sediment of a eutrophic reservoir, a novel salmon-pink-pigmented bacterium, designated NJ-44^T, was isolated. A polyphasic study suggested that strain NJ-44^T was most closely related to members of the genera *Terrimonas*, *Niabella* and *Niastella* of the phylum *Bacteroidetes*. Xie & Yokota (2006) described the genus *Terrimonas* to group the novel species *Terrimonas lutea* and *Flavobacterium ferrugineum*, a Gram-negative, aerobic, non-motile, soil organism. The genus *Terrimonas* has recently been included in the family *Crenotrichaceae* along with its close relative *Chitinophaga* (Garrity *et al.*, 2007). The other two recently described genera, *Niabella* and *Niastella*, were proposed by Kim *et al.* (2007) and Weon *et al.* (2006), respectively, for several soil isolates. They include *Niabella aurantiaca*, *Niastella yeongjuensis* and *Niastella koreensis*. In this study, polyphasic results showed that strain NJ-44^T represents a novel genus and species in the phylum *Bacteroidetes* related to, but distinct from, members of the genera *Terrimonas*, *Niabella* and *Niastella*.

Strain NJ-44^T was isolated from sediment of the seriously eutrophic Guanting Reservoir in Beijing (China). Characteristics of the sampled sediment 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, serially diluted sediment samples were spread onto plates of sediment steep medium prepared as follows: 400 g sediment was boiled in 1 l distilled water for 10 min and 0.5 g peptone, 0.5 g yeast extract and 15 g agar were then 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-44^T was picked and 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; K₂HPO₄, 0.3 g; MgSO₄ · 7H₂O, 0.05 g; distilled water 1 l; pH 7.2).

Cell morphology was observed under an Olympus light microscope at ×1000 with cells grown on R2A agar for 2 days at 28 °C. Growth at 4, 18, 22, 28, 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.*

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NJ-44^T is EF407879.

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.

(2002). Motility was investigated using the hanging drop technique with fresh cells in R2A broth. Utilization of carbon and energy sources was investigated in a basal medium containing (1^{-1}) 1 g $(\text{NH}_4)_2\text{HPO}_4$, 0.1 g yeast extract, 0.3 g K_2HPO_4 and 0.05 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, pH 7.2. Carbon substrates were added at a concentration of 0.5 % (w/v). All other biochemical tests were carried out according to Dong & Cai (2001). Cells grown in R2A broth for 3 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) according to the manufacturer's instructions. 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 phenotypic characteristics of strain NJ-44^T are given in the species description and in Table 1.

The 16S rRNA gene of strain NJ-44^T was amplified by PCR using the bacterial universal primers 27F and 1495R (Ying *et al.*, 2007) and a 1458 bp sequence was obtained with an ABI 3730 XL 96-capillary sequencer (Applied Biosystems).

The identification of 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). Further analysis performed using MEGA version 3.1 (Kumar *et al.*, 2004) showed that strain NJ-44^T was related to *Terrimonas ferruginea*, *Terrimonas lutea*, *Niabella aurantiaca*, *Niastella yeongjuensis* and *Niastella koreensis*, with 88.9–90.8 % 16S rRNA gene sequence similarity to the type strains. Phylogenetic trees were constructed with MEGA version 3.1 after multiple alignment of data by CLUSTAL W (Thompson *et al.*, 1994). With three different algorithms (neighbour-joining, minimum-evolution and maximum-parsimony), strain NJ-44^T clustered with members of the genera *Niabella* and *Terrimonas*. The neighbour-joining tree is shown in Fig. 1. The topology of the tree was evaluated by using Kimura's two-parameter calculation model (Kumar *et al.*, 2004) based on 1000 replicates. As shown in Fig. 1, strain NJ-44^T, *Niabella aurantiaca* R2A15-11^T, *T. ferruginea* ATCC 13524^T and *T. lutea* DY^T formed a branch that shared less than 90 % sequence similarity with members of the genera *Niastella* and *Chitinophaga*, though similarity between the latter was also about 90 %. The minimum-evolution and

Table 1. Differential characteristics of strain NJ-44^T and type strains of related species

Strains: 1, *Sediminibacterium salmoneum* gen. nov., sp. nov. NJ-44^T; 2, *Terrimonas ferruginea* IAM 15098^T; 3, *Terrimonas lutea* IAM 15284^T; 4, *Niabella aurantiaca* R2A15-11^T; 5, *Niastella yeongjuensis* DSM 17621^T; 6, *Niastella koreensis* DSM 17620^T; 7, *Chitinophaga pinensis* ACM 2034^T. Data for reference strains were taken from Xie & Yokota (2006), Kim *et al.* (2007), Weon *et al.* (2006) and Kämpfer *et al.* (2006). +, Positive; –, negative; w, weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Pigmentation*	SP	SR	Y	O	M	LY	Y
Production of:							
Flexirubin-type pigments	–	ND	ND	+	–	–	ND
H ₂ S	+	–	–	ND	ND	ND	–
Oxidase	+	+	+	–	+	–	+
Catalase	+	w	w	+	–	–	+
β-Galactosidase	+	+	–	+	+	+	ND
Urease	–	–	–	–	–	–	+
Gliding motility	+	–	–	–	+	+	+
Nitrate reduction	–	+	+	–	–	–	ND
Growth at 37 °C	+	+	+	ND	–	+	+
Highest NaCl tolerance (%)	1.0	1.0	1.0	3.0	ND	ND	ND
Hydrolysis of:							
Chitin	–	–	–	–	+	+	+
CM-cellulose	–	ND	ND	–	+	+	ND
Casein	–	ND	ND	+	+	+	ND
Assimilation of:							
Arabinose	–	–	+	+	–	–	+
Trehalose	+	–	+	ND	ND	ND	ND
D-Xylose	–	+	–	ND	ND	ND	ND
Melibiose	+	–	+	+	–	–	–
Sucrose	+	–	+	+	–	–	+
Rhamnose	–	+	–	+	–	–	+
DNA G + C content (mol%)	40.6	48.9	47.2	45.0	44.3	45.8	45.2

*LY, Light yellow; M, milky; O, orange; SP, salmon pink; SR, salmon red; Y, yellow.

Fig. 1. Neighbour-joining tree based on the 16S rRNA gene sequences of strain NJ-44^T and members of the genera *Terrimonas*, *Niabella*, *Niastella* and *Chitinophaga*. Numbers at nodes indicate bootstrap percentages >50 % from 1000 replicates. Bar, 0.01 substitutions per nucleotide position.

maximum-parsimony trees (available as Supplementary Fig. S1 in IJSEM Online) showed essentially the same topology.

The major cellular fatty acids of strain NJ-44^T were iso-C_{15:1} G, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} 3-OH, iso-C_{17:0} 3-OH, anteiso-C_{15:1} A and iso-C_{15:0} 3-OH, clearly distinguishing it from *Terrimonas* and *Niabella* species (Table 2).

The DNA G + C content of strain NJ-44^T was 40.6 mol%, well below those of *Terrimonas* species (47.2–48.9 mol%; Xie & Yokota, 2006) and *Niabella aurantiaca* (45.0 mol%; Kim *et al.*, 2007).

Data from 16S rRNA gene sequencing studies, cellular fatty acid composition and DNA G + C content showed that strain NJ-44^T cannot be assigned to the genera *Terrimonas* or *Niabella*, although they represent its closest phylogenetic neighbours. On the basis of phylogenetic and phenotypic data, it is proposed that strain NJ-44^T represents a new genus and species, for which the name *Sediminibacterium salmoneum* gen. nov., sp. nov. is proposed.

Description of *Sediminibacterium* gen. nov.

Sediminibacterium (Se'di.mi.ni.bac.te'ri.um. L. n. *sedimen*-*inis* sediment; L. neut. n. *bacterium* a rod; N.L. neut. n. *Sediminibacterium* a rod from sediment).

Table 2. Cellular fatty acid compositions of strain NJ-44^T and type strains of related species

Strains: 1, *Sediminibacterium salmoneum* gen. nov., sp. nov. NJ-44^T; 2, *Terrimonas ferruginea* IAM 15098^T; 3, *Terrimonas lutea* IAM 15284^T; 4, *Niabella aurantiaca* R2A15-11^T; 5, *Niastella yeongjuensis* DSM 17621^T; 6, *Niastella koreensis* DSM 17620^T; 7, *Chitinophaga pinensis* ACM 2034^T. Data for reference strains were taken from Xie & Yokota (2006), Kim *et al.* (2007), Weon *et al.* (2006) and Kämpfer *et al.* (2006). Some of the strains listed were grown under different culture conditions. Values are percentages of total fatty acids; fatty acids amounting to less than 1 % of total fatty acids in all strains studied are not listed. tr, Traces (<1 %).

Fatty acid	1	2	3	4	5	6	7
Unknown 13.565	1.5	1.3	3.2				2.6
Unknown 16.582	tr	1.3	1.8	1.2	1.6	1.4	1.1
iso-C _{14:0}	2.5	1.1	4.3				
iso-C _{15:1} G	24.1			22.3	14.7	15.6	
iso-C _{15:1}		26.2	21.8				
anteiso-C _{15:1} A	7.4						
iso-C _{15:0}	17.5	28.4	34.8	33.7	30.6	26.8	30.4
anteiso-C _{15:0}	9.5	tr		1.6	3.3	4.9	
C _{15:0}	tr	2.7	tr				tr
iso-C _{16:0}	1.1	1.4				1.0	
C _{16:1} ω11c							1.9
C _{16:1} ω5c	tr				1.2		33.2
C _{16:0}	1.2	1.7	1.1	3.5	2.9	2.6	4.2
iso-C _{15:0} 3-OH	7.4	2.2	2.3	2.9	1.9	1.3	3.1
C _{15:0} 2-OH	1.9	tr	tr				
iso-C _{16:0} 3-OH	7.7	tr					tr
C _{16:0} 3-OH	1.8	2.5	3.3	2.4	2.3	1.3	1.2
iso-C _{17:0} 3-OH	7.7	15.3	14.2	15.5	27.1	29.4	11.5
C _{17:0} 2-OH	2.2				2.6	3.5	
C _{17:0} 3-OH						1.2	
iso-C _{17:0}						1.6	tr
Summed feature 3*	tr	11.2	14.9	10.6	5.3	4.3	7.7

*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}ω7c.

Cells are strictly aerobic, Gram-negative rods. Oxidase- and catalase-positive. Flexirubin-type pigments are absent. The major cellular fatty acids are iso-C_{15:1} G, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0} 3-OH, iso-C_{17:0} 3-OH, anteiso-C_{15:1} A and iso-C_{15:0} 3-OH. The type species is *Sediminibacterium salmoneum*. Phylogenetically related to the genera *Terrimonas* and *Niabella* in the phylum *Bacteroidetes*.

Description of *Sediminibacterium salmoneum* sp. nov.

Sediminibacterium salmoneum (sal.mo.ne'um. L. n. *salmo* -onis salmon; L. adj. suff. -eus -a -um suffix used with various meanings; N.L. neut. adj. *salmoneum* salmon-coloured).

The characteristics are the same as those given in the genus description. Cells are single rods, 0.2–0.3 µm in diameter and 1.0–1.2 µm long, with gliding motility. Colonies on R2A agar are salmon-pink, circular, about 0.5–1 mm in diameter and non-spreading after 3 days of incubation at 28 °C. Growth occurs at 18–37 °C (optimum, 22–28 °C), at pH 6.0–7.5 (optimum, 7.0) and with up to 1 % NaCl (optimum growth in the absence of NaCl). No growth occurs on TSA, NA, MA or PYG agar. H₂S is produced. Nitrate is not reduced. *o*-Nitrophenyl β-D-galactopyranoside (ONPG) is hydrolysed. Gelatin is degraded but pectin, chitin, Tween 80, CM-cellulose and casein are not. Growth and acid formation occur with raffinose, salicin, lactose, melezitose, D-mannose, trehalose, melibiose, glucose, sucrose and maltose. Of the latter, raffinose, trehalose and sucrose are the best carbon sources. Mannitol, D-galactose, L-rhamnose, D-xylose, D-sorbose, D-sorbitol, D-fructose, inulin, D-arabinose, L-arabinose, D-ribose, inositol, citric acid, citrate, sorbic acid, succinate, glycerol, aspartic acid and L-tyrosine are not utilized. Alkaline phosphatase and weak lipase activities are present, but urease, tryptophan deaminase and arginine dihydrolase activities are absent. The major fatty acids (>5 %) are iso-C_{15:1} G (24.1 %), iso-C_{15:0} (17.5 %), anteiso-C_{15:0} (9.5 %), iso-C_{16:0} 3-OH (7.7 %), iso-C_{17:0} 3-OH (7.7 %), anteiso-C_{15:1} A (7.4 %) and iso-C_{15:0} 3-OH (7.4 %). The G+C content of the genomic DNA of the type strain is 40.6 mol%.

The type strain is NJ-44^T (=CGMCC 1.6845^T =NBRC 103935^T), isolated from the sediment of Guanting Reservoir in Beijing, China.

Acknowledgements

This work was supported by a project from the Chinese National Natural Science Foundation (no. 30670071), the Hi-tech Research and Development Program (863 Program) of China (no. 2006AA10A213) and the Taihu Project (no. BK2007741) from Jiangsu Science and Technology Department. We make a grateful acknowledgement to the Associate Editor for his kind help in modification of the manuscript. We are indebted to Dr Jean Euzéby (Ecole Nationale Vétérinaire, Toulouse, France) for his kind assistance in Latin nomenclature.

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