Lacibacter cauensis gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic lake

Jian-Hang Qu, Hong-Li Yuan, Jin-Shui Yang, Hai-Feng Li and Nan Chen

Correspondence

Hong-Li Yuan

hlyuan@cau.edu.cn

State Key Lab for Agrobiotechnology, Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture, College of Biological Sciences, China Agricultural University, Beijing 100093, PR China

A novel strain, NJ-8^T, was isolated from sediment of the eutrophic Taihu Lake (China) and was subjected to a taxonomic study by using a polyphasic approach. The novel strain was aerobic and formed orange-pigmented colonies on R2A agar. Cells were Gram-negative single rods that were motile by means of gliding. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain NJ-8^T belonged to the phylum *Bacteroidetes*, with *Niastella yeongjuensis* DSM 17621^T (92.3 % gene sequence similarity) and *Niastella koreensis* DSM 17620^T (92.0 % similarity) as its closest relatives. The major fatty acids (>5 % of the total) were iso-C_{15:0} (27.8 %), iso-C_{15:1} G (15.4 %), iso-C_{17:0} 3-OH (14.5 %) and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 13.9 %). The genomic DNA G + C content was 46.6 mol%. Based on molecular and phenotypic data, strain NJ-8^T is considered to represent a novel species of a new genus, for which the name *Lacibacter cauensis* gen. nov., sp. nov. is proposed. The type strain of the type species of the genus, *Lacibacter cauensis*, is NJ-8^T (=CGMCC 1.7271^T=NBRC 104930^T).

During the course of a study on the bacterial diversity in sediment from a eutrophic lake, a novel orange-pigmented bacterium, designated strain NJ-8^T, was isolated. On the basis of phylogenetic analysis, this strain was found to be most closely related to members of the genera *Niastella*, *Terrimonas* and *Flavisolibacter*, within the phylum *Bacteroidetes*. The genus *Niastella* was described by Weon *et al.* (2006) on the basis of two novel species, namely *Niastella yeongjuensis* and *Niastella koreensis*. Members of this genus are Gram-negative, aerobic, non-flagellated, gliding and filamentous micro-organisms. The genus *Terrimonas* was proposed by Xie & Yokota (2006) with the description of *Terrimonas lutea* and the reclassification of [*Flavobacterium*] *ferrugineum* as *Terrimonas ferruginea*. The genus *Terrimonas* has recently been included in the family *Crenotrichaceae* along with the closely related genus *Chitinophaga* (Garritty *et al.*, 2007). The genus *Flavisolibacter* was proposed by Yoon & Im (2007) with the description of *Flavisolibacter ginsengiterrae* and *Flavisolibacter ginsengisoli*. In the present study, data from a polyphasic investigation show that strain NJ-8^T represents a novel species of a new genus in the phylum

Bacteroidetes related to, but distinct from, members of the genera *Niastella*, *Terrimonas* and *Flavisolibacter*.

Strain NJ-8^T was isolated from the surface layer sediment of the highly eutrophic Taihu Lake in Jiangsu Province, China. This sediment was collected on 18 November 2007. For isolation, serially diluted sediment samples were spread onto plates of sediment steep medium prepared as follows: 400 g sediment was boiled in a litre of distilled water for 10 min; 0.5 g peptone, 0.5 g yeast extract and 15 g agar were then added to a litre of supernatant (the pH was not adjusted). Plates were incubated at 28 °C for 7 days. A single colony of strain NJ-8^T was picked and subcultured on modified R2A media (per litre distilled water: 0.5 g yeast extract, 0.5 g polypeptone, 0.5 g casein hydrolysate, 0.5 g glucose, 0.5 g soluble starch, 0.3 g K₂HPO₄ and 0.05 g MgSO₄·7H₂O; pH 7.2).

Cell morphology was observed under an Olympus light microscope at ×1000 magnification with cells grown on R2A agar for 2 days at 28 °C. Growth at 4, 20, 28, 37 and 50 °C and at pH 5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 was assessed after 7 days incubation. The pH of the 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 7 days incubation. Growth on nutrient agar (NA), which was prepared in the laboratory according to the composition of Difco media, and LB agar (Sambrook & Russell, 2001) was evaluated at 28 °C for 7 days. Flexirubin-type pigments were detected

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NJ-8^T is EU521690.

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

by flooding a small mass of bacterial cells with 20 % KOH according to the minimal standards described by Bernardet *et al.* (2002). Motility was investigated by using the hanging-drop technique with fresh cells in R2A broth. Utilization of carbon and energy sources was investigated in basal medium containing (per litre distilled water): 1 g (NH₄)₂SO₄, 0.3 g K₂HPO₄, 0.05 g MgSO₄·7H₂O and 0.1 g yeast extract (pH 7.2). Carbon substrates were added at a concentration of 0.5 % (w/v). After 7 days incubation, bromothymol blue (0.1 %, v/v) was used as an indicator to test for the production of acids. Hydrolysis of starch (0.2 %, w/v), casein (1 %, w/v), pectin (0.5 %, w/v), Tween 80 (1 %, v/v), L-tyrosine (0.5 %, w/v) and egg-yolk (3 %, v/v) was tested by using R2A as the basal medium (Yi & Chun, 2006). The Gram reaction, oxidase and catalase activities, methyl-red reaction, glucose oxidation/fermentation, nitrate reduction, denitrification, production of H₂S, gelatin liquefaction, and lipase, lecithinase, β -galactosidase, urease, DNase and tryptophan deaminase activities were investigated according to the methods of Dong & Cai (2001).

Cells grown in R2A broth for 2 days at 28 °C were used for cellular fatty acid analysis. Fatty acid methyl esters were prepared and identified by using the Sherlock Microbial Identification System (MIDI) according to the manufacturer's instructions. Genomic DNA was extracted accord-

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

The phenotypic characteristics of strain NJ-8^T are given in the genus and species descriptions below and in Table 1. The new isolate could be distinguished clearly from related taxa based on cell length, catalase activity and assimilation of carbohydrates.

PCR amplification of the 16S rRNA gene was carried out by using the bacterial universal primers 27F (5'-GAGAGTTTGATCCTGGCTCAG-3', *E. coli* position 27–46) and 1495R (5'-CTACGGCTACCTGTTACGA-3', *E. coli* position 1476–1495). A nearly complete sequence (1412 bp) was obtained with an ABI 3730 XL 96-capillary sequencer (Applied Biosystems). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved by using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007). Results showed that strain NJ-8^T was most closely related to the type strains of *N. yeongjuensis*, *N. koreensis*, *T. ferruginea*, *F. ginsengisoli*, *F. ginsengiterrae* and *T. lutea* (92.3–90.8 % 16S rRNA gene sequence similarity). Phylogenetic trees were constructed via MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the data

Table 1. Differential phenotypic characteristics between strain NJ-8^T and the type strains of related species

Strains: 1, NJ-8^T; 2, *N. yeongjuensis* DSM 17621^T; 3, *N. koreensis* DSM 17620^T; 4, *T. ferruginea* IAM 15098^T; 5, *T. lutea* IAM 15284^T; 6, *F. ginsengisoli* Gsoil 643^T; 7, *F. ginsengiterrae* Gsoil 492^T. Data for reference strains are from Weon *et al.* (2006), Kämpfer *et al.* (2006) and Yoon & Im (2007). +, Positive; –, negative; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Pigmentation	Orange	Milky white	Light yellow	Salmon red	Yellow	Yellow	Yellow
Cell length (µm)	1.0–2.0	10–40	10–50	0.6–4.0	1–2	0.3–6.0	0.3–1.0
Production of:							
H ₂ S	+	ND	ND	–	–	–	–
Oxidase	+	+	–	+	+	–	+
Catalase	+	–	–	+	w	+	–
β -Galactosidase	+	+	+	+	–	+	+
Gliding motility	+	+	+	–	–	–	–
Nitrate reduction	–	–	–	+	+	–	–
Growth at 37 °C	+	–	+	+	+	+	–
Highest NaCl tolerance (%)	1.0	ND	ND	1.0	1.0	1.0	3.0
Hydrolysis of:							
Carboxymethylcellulose	–	+	+	ND	ND	ND	ND
Casein	+	+	+	ND	ND	–	–
Starch	+	–	–	+	ND	–	–
Assimilation of:							
L-Arabinose	–	–	–	–	+	–	+
Trehalose	+	ND	ND	–	+	ND	ND
D-Xylose	–	ND	ND	+	–	ND	ND
Melibiose	+	–	–	–	+	–	+
Sucrose	+	–	–	–	+	+	+
L-Rhamnose	+	–	–	+	–	–	+
DNA G + C content (mol%)	46.6	44.3	45.8	48.9	47.2	42.7	43.0

by CLUSTAL W (Thompson *et al.*, 1994). Three different algorithms (neighbour-joining, minimum-evolution and maximum-parsimony) were used and the topology of the tree was evaluated by using the Kimura two-parameter calculation model (Kumar *et al.*, 2004) based on 1000 replicates. In the neighbour-joining tree (Fig. 1), strain NJ-8^T, *N. yeongjuensis* DSM 17621^T, *N. koreensis* DSM 17620^T, *T. ferruginea* IAM 15098^T, *F. ginsengisoli* Gsoil 643^T, *F. ginsengiterrae* Gsoil 492^T and *T. lutea* IAM 15284^T formed a single cluster. The minimum-evolution and maximum-parsimony trees showed essentially the same topology (see Supplementary Fig. S1 in IJSEM Online).

The major cellular fatty acids of strain NJ-8^T were iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), a profile distinct from those of species of the genera *Niastella* and *Terrimonas* (Table 2).

The DNA G+C content of strain NJ-8^T was 46.6 mol%, different from that of recognized species of the genera *Niastella* (44.3–45.8 mol%; Weon *et al.*, 2006) and *Terrimonas* (47.2–48.9 mol%; Xie & Yokota, 2006), as indicated in Table 1.

On the basis of phylogenetic and phenotypic data, strain NJ-8^T is considered to represent a novel species of a new genus in the phylum *Bacteroidetes*, for which the name *Lacibacter cauensis* gen. nov., sp. nov. is proposed.

Description of *Lacibacter* gen. nov.

Lacibacter (La.ci.bac'ter. L. n. *lacus* lake; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Lacibacter* rod-shaped bacterium from lake sediment).

Cells are aerobic, Gram-negative rods. Oxidase- and catalase-positive. Flexirubin-type pigments are absent. The major cellular fatty acids are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). The type species is *Lacibacter cauensis*. Phylogenetically related to the genera *Niastella*, *Terrimonas* and *Flavisolibacter* in the phylum *Bacteroidetes*.

Description of *Lacibacter cauensis* sp. nov.

Lacibacter cauensis (cau.en'sis. N.L. masc. adj. *cauensis* pertaining to CAU, the acronym of the China Agricultural University, where the taxonomic studies on the type strain were performed).

Has the following characteristics in addition to those given in the genus description. Cells are single rods about 0.4–0.6 μm in diameter and 1.0–2.0 μm in length, and are motile by means of gliding. Colonies grown on R2A agar plates are circular, orange and about 0.5–1.0 mm in diameter after 7 days incubation at 28 °C. Growth occurs at 20–37 °C (optimum, 28–37 °C), at pH 5.5–8.0 (optimum, pH 7.0) and in the presence of 0–1 % NaCl (optimum, 0 %). Grows on NA (weakly) but not on LB agar. H₂S is produced. Negative for denitrification and reduction of nitrate. ONPG is hydrolysed. Methyl-red reaction is positive. Positive for hydrolysis of starch and casein. Negative reactions are observed for the hydrolysis of carboxymethylcellulose, pectin, Tween 80, urea and DNA. Tyrosine is weakly degraded, accompanied by production of a dark red pigment. Gelatin is liquefied slowly. Positive for oxidation of glucose and weakly positive for fermentation of glucose. Growth and acid formation occur with many carbohydrate substrates: maltose, sucrose, D-glucose, salicin, lactose, D-fructose, melezitose, D-galactose, raffinose, D-mannose, mannitol, L-rhamnose, trehalose, melibiose, *myo*-inositol, D-sorbitol and sodium succinate. Growth is strongest with maltose, sucrose and D-glucose. Unable to utilize D-xylose, D-sorbose, D-sodium gluconate, inulin, D-arabinose, L-arabinose, D-ribose, trisodium citrate dehydrate, L-asparagine, *n*-butanol or glycerol. Lecithinase and β-galactosidase are present but urease, tryptophan deaminase, DNase and lipase are absent. The major fatty acids are iso-C_{15:0}, iso-C_{15:1} G, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH).

The type strain, NJ-8^T (=CGMCC 1.7271^T=NBRC 104930^T), was isolated from the sediment of Taihu Lake in Jiangsu Province, China. The G+C content of the genomic DNA of the type strain is 46.6 mol%.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain NJ-8^T among related species. Numbers at nodes indicate bootstrap percentages (based on 1000 replicates); only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid composition (%) of strain NJ-8^T and related species

Strains: 1, NJ-8^T; 2, *N. yeongjuensis* DSM 17621^T; 3, *N. koreensis* DSM 17620^T; 4, *T. ferruginea* IAM 15098^T; 5, *T. lutea* IAM 15284^T; 6, *F. ginsengisoli* Gsoil 643^T; 7, *F. ginsengiterrae* Gsoil 492^T. Data for reference strains are from Weon *et al.* (2006), Kämpfer *et al.* (2006) and Yoon & Im (2007). Fatty acids amounting to <1 % of the total fatty acids in all strains studied are not listed. tr, trace (<1 %).

Fatty acid	1	2	3	4	5	6	7
Unknown 13.565	4.2			1.3	3.2		
iso-C _{14:0}	tr			1.1	4.3		
iso-C _{15:1} G	15.4	14.7	15.6				
iso-C _{15:1}				26.2	21.8	4.4	5.3
iso-C _{15:0}	27.8	30.6	26.8	28.4	34.8	31.2	45.9
anteiso-C _{15:0}	tr	3.3	4.9	tr		5.0	5.9
C _{15:0}	4.7			2.7	tr		
iso-C _{16:0}	tr		1.0	1.4			
C _{16:1} ω5 <i>c</i>	1.6	1.2					
C _{16:0}	2.4	2.9	2.6	1.7	1.1	7.5	5.5
iso-C _{15:0} 3-OH	2.7	1.9	1.3	2.2	2.3	1.0	1.4
Unknown 16.582	1.5	1.6	1.4	1.3	1.8		
iso-C _{17:0}	1.2		1.6			9.1	3.1
anteiso-C _{17:0}						1.0	
iso-C _{17:1} ω9 <i>c</i>							1.3
C _{16:0} 10-methyl						2.6	
C _{16:0} 2-OH						2.9	
C _{16:0} 3-OH	tr	2.3	1.3	2.5	3.3		
C _{18:1} ω7 <i>c</i>	1.9						
C _{18:0}	tr					2.0	
iso-C _{17:0} 3-OH	14.5	27.1	29.4	15.3	14.2	11.8	13.7
C _{17:0} 2-OH		2.6	3.5				
C _{17:0} 3-OH	tr		1.2				
Summed feature:*							
2						2.5	2.7
3	13.9	5.3	4.3	11.2	14.9		
4	1.2					6.2	10.5
5						3.1	1.4
6						1.7	
7						1.2	

*Summed features are combinations of fatty acids that cannot be separated by the MIDI system. Summed feature 2 comprises C_{12:0} aldehyde, C_{14:0} 3-OH and/or iso-C_{16:1}; summed feature 3 comprises C_{16:1}ω7*c* and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 5 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 6 comprises C_{18:2}ω6,9*c* and/or anteiso-C_{18:0}; and summed feature 7 comprises C_{18:1}ω7*c*, C_{18:1}ω9*t* or C_{18:1}ω12*t* or any combination of these isomers.

Acknowledgements

This work was supported by the Taihu Project (No. BK2007741) from Jiangsu Science and Technology Department, a project from the Chinese National Natural Science Foundation (No. 30670071) and the China Postdoctoral Science Foundation (No. 20070420432).

References

Bernardet, J. F., Nakagawa, Y. & Holmes, B. (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.

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.

De Ley, J. (1970). Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxy-ribonucleic acid. *J Bacteriol* **101**, 738–754.

Dong, X.-Z. & Cai, M.-Y. (2001). *Determinative Manual for Routine Bacteriology*. Beijing: Scientific Press.

Garrity, G., Lilburn, T., Cole, J., Harrison, S., Euzéby, J. & Tindall, B. (2007). Part 11 – The Bacteria: Phyla ‘Planctomycetes’, ‘Chlamydiae’, ‘Spirochaetes’, ‘Fibrobacteres’, ‘Acidobacteria’, ‘Bacteroidetes’, ‘Fusobacteria’, ‘Verrucomicrobia’, ‘Dictyoglomi’, ‘Gemmatomonadetes’,

and 'Lentisphaerae'. In *The Taxonomic Outline of Bacteria and Archaea*, TOBA release 7.7. doi: 10.1601/toba.v7i7.188

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.

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Sambrook, J. & Russell, D. W. (2001). *Molecular Cloning, a Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment

through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

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.

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.

Yi, H. & Chun, J. (2006). *Flavobacterium weaverense* sp. nov. and *Flavobacterium segetis* sp. nov., novel psychrophiles isolated from the Antarctic. *Int J Syst Evol Microbiol* **56**, 1239–1244.

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.