Novosphingobium sediminicola sp. nov. isolated from freshwater sediment

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A yellow-pigmented, Gram-negative, short rod-shaped, non-motile and non-spore-forming bacterial strain, designated HU1-AH51^T, was isolated from freshwater sediment and was characterized using a polyphasic approach, in order to determine its taxonomic position. On the basis of 16S rRNA gene sequence similarity, strain HU1-AH51^T was shown to belong to the genus *Novosphingobium*, showing the highest level of sequence similarity with respect to *Novosphingobium resinovorum* NCIMB 8767^T (96.0 %), *Novosphingobium naphthalenivorans* TUT562^T (96.0 %) and *Novosphingobium panipatense* SM16^T (96.0 %). Strain HU1-AH51^T had a genomic DNA G+C content of 62.6 mol% and Q-10 as the predominant respiratory quinone. Furthermore, the major polyamine component (spermidine) in the cytoplasm and the presence of sphingoglycolipids suggested that strain HU1-AH51^T belongs to the family *Sphingomonadaceae*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain HU1-AH51^T represents a novel species of the genus *Novosphingobium*, for which the name *Novosphingobium sediminicola* sp. nov. is proposed. The type strain is HU1-AH51^T (=LMG 24320^T =KCTC 22311^T).

The genus *Novosphingobium* was proposed by Takeuchi *et al.* (2001) to accommodate six species of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990), because the genus *Sphingomonas* represented a broad range of heterogeneous species with respect to physiology, phylogenetics and ecology. The nomenclatural system was replaced by the proposal of Takeuchi *et al.* (2001) and has been generally accepted and used (Kämpfer *et al.*, 2002; Fujii *et al.*, 2003; Sohn *et al.*, 2004; Liu *et al.*, 2005; Tiirola *et al.*, 2005; Addison *et al.*, 2007; Lim *et al.*, 2007; Suzuki & Hiraishi, 2007; Glaeser *et al.*, 2009; Gupta *et al.*, 2009; Yuan *et al.*, 2009). At the time of this writing, the genus *Novosphingobium* comprised 18 recognized species. *Novosphingobium capsulatum* is the type species of the genus *Novosphingobium*.

Sediment from a freshwater lake was investigated for bacterial culturability and diversity (Lim *et al.*, 2008), and one novel isolate, HU1-AH51^T, was found to be phylogenetically related to members of the genus *Novosphingobium*. Sediment samples were collected from a littoral zone of Lake Hakha (36° 16′ 60″ N 127° 18′ 32″ E), a typical freshwater lake in South Korea, and subjected to dilution-to-extinction cultivation (Button *et al.*, 1993) in 96-well plates, wherein cells were enriched on unsupplemented lake water media of low nutritional status for 3 months, followed by the detection of extinction wells, as previously described by Lim *et al.* (2008). Single colonies were isolated on 1/10 R2A agar plates and then transferred to R2A agar cultivated at 30 °C. Cells were maintained in a glycerol suspension (20 %, w/v) with R2A broth at −70 °C.

Colonies were directly applied to 16S rRNA gene PCR using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991) and amplified fragments were then sequenced using the primers 27F and 1100R (Lane, 1991), 341F and 534R (Muyzer *et al.*, 1993) and 907F (Lane *et al.*, 1985). 16S rRNA gene sequences of strain HU1-AH51^T were assembled with Seqman II 5.0 (DNASTAR) (Swindell & Plasterer, 1997) and compared with all 16S rRNA gene sequences available in the GenBank database. Alignment of 22 sequences, including the type strains of species of the genus *Novosphingobium*, was carried out with CLUSTAL X (Thompson *et al.*, 1997). Gaps at the 5′ and 3′ ends and ambiguous bases were removed from the alignment using BioEdit (Hall, 1999). Phylogenetic trees based on comparison of 1336 bases were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HU1-AH51^T is FJ177534.

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reconstructed by the neighbour-joining (Saitou & Nei, 1987) and the maximum-parsimony (Fitch, 1971) algorithms using MEGA version 3.1 (Kumar *et al.*, 2004). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) and bootstrap values were based on 1000 replications (Felsenstein, 1985) for the neighbour-joining and maximum-parsimony methods. The maximum-likelihood method, using the program DNAML under the assumption that the transition/transversion ratio was 2.000 in the PHYLIP software package (Felsenstein, 1989), was also used for phylogenetic analysis. Chromosomal DNA was extracted and purified using Qiagen Genomic-tip system 100/G for determination of G+C content, which was then measured as described by Mesbah *et al.* (1989) using a reverse-phase HPLC (Younglin Instrument Co., Republic of Korea).

The Gram reaction was performed by using the bioMérieux Gram stain kit and the non-staining method as described by Buck (1982). Cell morphology was observed under a Nikon light microscope ($\times$1000), using cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined by means of bubble production in 3 % (v/v) H_2O_2. Oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Growth at different temperatures (4, 10, 18, 24, 30, 37 and 45 °C) and pH (4.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days of incubation. Salt tolerance was tested on modified R2A medium (l^{-1}: 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4, 0.05 g $MgSO_4$, 0.05 g $CaCl_2$) supplemented with 1–10 % (w/v) NaCl and 50 mM sodium phosphate buffer (pH 7.0) after 5 days of incubation. Growth on nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco) was also evaluated at 30 °C.

Physiological characteristics were determined using ID 32GN, API 20NE and API ZYM strips according to the manufacturer's instructions (bioMérieux). Results of API tests were read after 2 days of incubation except for API ZYM (4 h). Anaerobic growth was tested on R2A broth containing thioglycolate (1 g l^{-1}) in a serum bottle with the headspace substituted with nitrogen gas. DNase activity was detected on DNase test agar (Scharlau) by flooding culture plates with 1 M HCl. Hydrolysis of starch, chitin, aesculin and skim milk were examined according to Atlas (2004). Activities for degradation of cellulose and xylan were studied according to Ten *et al.* (2004). Isoprenoid quinones were determined as described by Komagata & Suzuki (1987) using Sep-Pak Vac silica cartridges (Waters). Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI) after 2 days of incubation at 30 °C on R2A. The fatty acids analysed by GC (6890; Hewlett Packard), were identified by the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted using the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents: 5 % ethanolic molybdatophosphoric acid for total lipids, ninhydrin for aminolipids, molybdenum blue (Sigma) for phospholipids and α-naphthol-sulphuric acid reagent for glycolipids (Komagata & Suzuki, 1987). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Schenkel *et al.* (1995).

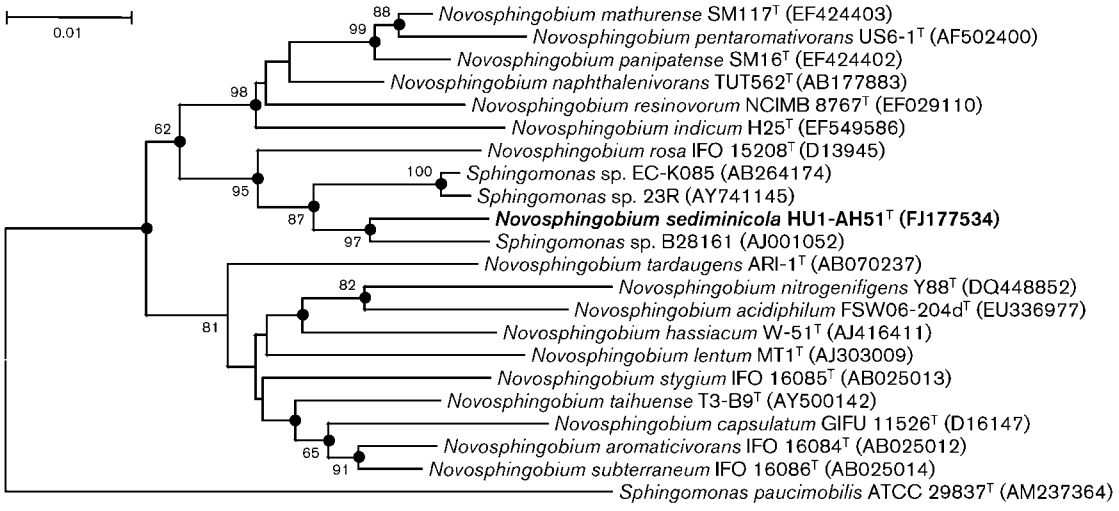

Fig. 1. Neighbour-joining phylogenetic tree, reconstructed from a comparative analysis of 16S rRNA gene sequences, showing the relationships between strain HU1-AH51^T and related species. Filled circles indicate generic branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

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The almost full-length 16S rRNA gene sequence of strain HU1-AH51^T determined in this study was 1387 bp in length. Sequence similarity values were estimated by using the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). The closest relatives to strain HU1-AH51^T were three uncharacterized isolates, *Sphingomonas* sp. B28161 (98.2 %), *Sphingomonas* sp. EC-K085 (97.5 %) and *Sphingomonas* sp. 23R (97.3 %), and the closest relatives representing recognized species were *Novosphingobium resinovorum* NCIMB 8767^T (96.0 %), *Novosphingobium naphthalenivorans* TUT562^T (96.0 %), *Novosphingobium panipatense* SM16^T (96.0 %), *Novosphingobium rosa* IFO 15208^T (95.9 %) and *Novosphingobium mathurense* SM117^T (95.9 %). Lower sequence similarities (<95.7 %) were found with the other recognized species of the genus *Novosphingobium*. This relationship between strain HU1-AH51^T and other members of the genus *Novosphingobium* was confirmed in the neighbour-joining phylogenetic tree (Fig. 1) and the maximum-likelihood phylogenetic tree (Supplementary Fig. S1, available in IJSEM Online). Strain HU1-AH51^T and *Novosphingobium rosa* formed a distinct cluster within the genus *Novosphingobium*.

Strain HU1-AH51^T was Gram-staining negative, strictly aerobic, non-motile and rod-shaped. Colonies grown on R2A agar plates for 3 days were circular, smooth, convex, mucous, yellow and 1.0–1.5 mm in diameter. Catalase and oxidase reactions were positive. Strain HU1-AH51^T showed growth on R2A, NA and TSA medium. On R2A agar, strain HU1-AH51^T was able to grow at 18–37 °C with an optimal growth temperature of 30 °C. Cells could also be cultivated in R2A broth media with the addition of NaCl (0–1 %) and at pH 5.5–9.5 (optimum pH 7.5). The physiological characteristics of strain HU1-AH51^T are summarized in the species description and comparisons of selective characteristics with all type strains of the genus *Novosphingobium* are shown in Table 1.

The DNA G+C content of strain HU1-AH51^T was 62.6 mol%. The fatty acids of strain HU1-AH51^T (Table 2) mainly comprised $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (included

Table 1. Physiological and biochemical characteristics of strain HU1-AH51^T and type strains of species of the genus *Novosphingobium*

Strains: 1, *Novosphingobium sediminicola* sp. nov. HU1-AH51^T; 2, *N. rosa* DSM 7285^T; 3, *N. resinovorum* DSM 7478^T; 4, *N. naphthalenivorans* DSM 18518^T; 5, *N. capsulatum* DSM 30196^T (data for columns 1–5 from this study); 6, *N. lentum* MT1^T; 7, *N. tardaugens* JCM 11434^T; 8, *N. hassiacum* DSM 14552^T; 9, *N. stygium* SMCC B0712^T; 10, *N. subterraneum* SMCC B0478^T; 11, *N. aromaticivorans* SMCC F199^T; 12, *N. pentaromativorans* KCTC 10454^T (data for columns 6–12 from Tiirola *et al.*, 2005); 13, *N. indicum* H25^T; 14, *N. nitrogenifigens* Y88^T; 15, *N. taihuense* T3-B9^T (data for columns 13–15 from Yuan *et al.*, 2009); 16, *N. panipatense* SM16^T; 17, *N. mathurense* SM117^T (data for columns 16–17 from Gupta *et al.*, 2009); 18, *N. acidiphilum* FSW06-204d^T (data from Glaeser *et al.*, 2009). +, Positive; –, negative; (+), weakly positive; ND, not detected or not described. All strains showed negative results for assimilation of D-mannitol.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Hydrolysis of aesculin	+	–	–	–	–	–	–	–	+	+	+	+	–	–	+	–	+	+
Nitrate reduction	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	(+)	–	ND
Gelatin hydrolysis	–	–	–	–	+	–	–	–	–	+	–	–	+	–	–	–	–	ND
Indole production	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ND
Arginine dihydrolase	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	ND	ND	ND
Urease	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	ND	ND	ND
β-Galactosidase	–	–	–	–	+	–	–	–	+	+	+	–	–	–	ND	ND	ND	ND
Assimilation of:																		
D-Glucose	+	–	+	+	+	–	–	+	–	+	+	–	+	+	+	+	+	+
L-Arabinose	+	–	+	+	+	–	–	–	–	(+)	–	–	–	–	+	+	+	(+)
D-Mannose	+	–	–	–	+	–	–	–	–	+	+	–	+	+	+	+	+	+
Maltose	+	–	–	–	+	–	–	–	–	+	+	–	+	+	+	+	+	+
L-Proline	+	–	+	+	–	–	–	+	–	+	+	+	+	–	+	+	+	+
L-Rhamnose	+	–	–	–	+	–	–	–	–	+	+	+	–	–	+	–	+	+
Sucrose	+	–	–	–	+	–	–	+	–	+	+	+	–	+	+	(+)	+	+
N-Acetylglucosamine	+	–	–	–	+	–	–	–	–	–	+	–	–	+	–	ND	ND	ND
L-Alanine	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	ND	ND	ND
Malic acid	–	–	+	–	+	–	–	–	–	–	–	–	ND	+	ND	ND	ND	+
Lactic acid	–	+	–	–	–	–	+	–	–	–	–	–	ND	+	ND	ND	ND	–
L-Histidine	–	–	–	–	+	–	–	–	–	–	–	–	ND	+	ND	ND	ND	–
L-Serine	–	–	–	–	–	–	–	–	–	–	–	–	ND	ND	ND	+	–	ND
D-Sorbitol	–	–	–	–	–	–	–	–	–	–	–	–	ND	ND	ND	+	–	ND
Propionic acid	–	–	–	–	–	–	–	+	–	+	+	–	ND	ND	ND	(+)	+	ND
Malonate	–	–	–	–	–	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	–	(+)	ND

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Table 2. Cellular fatty acid profiles of strain HU1-AH51^T and related type strains of species of the genus *Novosphingobium*

Strains: 1, *Novosphingobium sediminicola* sp. nov. HU1-AH51^T; 2, *N. rosa* DSM 7285^T; 3, *N. resinovorum* DSM 7478^T; 4, *N. naphthalenivorans* DSM 18518^T; 5, *N. capsulatum* DSM 30196^T. Data for all strains are from this study and are based on the Microbial Identification System. Values shown are percentages of total fatty acids; major fatty acids are shown in bold. –, Not detected.

Fatty acid	1	2	3	4	5
$C_{14:0}$	2.75	3.68	2.94	0.88	1.53
$C_{15:0}$	–	–	–	2.28	1.05
$C_{16:0}$	**12.11**	**22.94**	**21.89**	**15.03**	**17.09**
$C_{16:1}$ $\omega5c$	2.12	–	1.22	0.98	–
$C_{17:1}$ $\omega6c$	–	–	–	8.19	1.12
$C_{17:1}$ $\omega8c$	–	–	–	1.01	–
$C_{18:1}$ $\omega5c$	3.33	–	6.74	3.44	4.34
$C_{17:0}$ cyclo	–	4.56	–	–	–
$C_{19:0}$ cyclo $\omega8c$	2.69	5.32	1.28	1.03	2.07
$C_{14:0}$ 2-OH	–	7.76	7.52	3.77	6.69
$C_{15:0}$ 2-OH	–	–	–	1.00	1.35
$C_{16:0}$ 2-OH	–	–	2.30	–	–
Summed features*					
4	**43.68**	**28.41**	**13.94**	9.18	4.35
7	**33.32**	**27.33**	**42.18**	**53.22**	**60.41**

* Summed feature 4 consists of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 7 consists of one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$.

within summed feature 4), one or more of $C_{18:1}\omega7c$, $C_{18:1}\omega9c$, $C_{18:1}\omega9t$ and $C_{18:1}\omega12t$ (included within summed feature 7) and $C_{16:0}$. The major polyamine component is spermidine (25.2 μmol dry weight g^{-1}), with lesser amounts of spermine (0.9 μmol dry weight g^{-1}). The major isoprenoid quinone was ubiquinone Q-10. The polar lipid profile of strain HU1-AH51^T was obtained (Supplementary Fig. S2). Cells contain phosphatidylethanolamine, phosphatidyldimethylethanolamine, phosphatidylglycerol, sphingoglycolipid and phosphatidylcholine as major polar lipids; they also contain unknown glycolipids (GL1–2) and other unknown lipids (L1–2). These chemotaxonomical data support the affiliation of strain HU1-AH51^T to the genus *Novosphingobium*.

All of the characteristics determined for strain HU1-AH51^T were in accordance with those for the genus *Novosphingobium*. However, the phylogenetic distances from recognized species of the genus *Novosphingobium* and the combination of unique phenotypic characteristics indicated that strain HU1-AH51^T is not affiliated to any recognized species of the genus *Novosphingobium*. Therefore, it is concluded that strain HU1-AH51^T represents a novel species of the genus *Novosphingobium*, for which the name *Novosphingobium sediminicola* sp. nov. is proposed.

Description of *Novosphingobium sediminicola* sp. nov.

Novosphingobium sediminicola [se.di.mi.ni.co′la. L. n. *sedimen –inis* sediment; L. suff. *–cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *sediminicola*, sediment-dweller, referring to the source of the type strain].

Cells are Gram-negative, strictly aerobic, non-motile, short rod-shaped, 0.8–1.0 μm in diameter and 1.2–1.5 μm in length. Catalase- and oxidase-positive. Grows at 18–37 °C, pH 5.5–9.5 and with 0–1 % (w/v) NaCl. Growth occurs on NA and TSA media. Nitrate is not reduced. Sphingoglycolipid is present. Spermidine is the major polyamine component. The major isoprenoid quinone is ubiquinone Q-10. Casein, cellulose, chitin, DNA, starch and xylan are not degraded. In API ZYM, 2-naphthyl phosphate (pH 8.5), 6-bromo-2-naphthyl β-D-glucopyranoside and naphthol-AS-BI-phosphate are hydrolysed, but the following substances are not: naphthol-AS-BI-β-D-glucuronide, 1-nitrophenyl-*N*-acetyl-β-D-glucosaminide, 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, 2-naphthyl phosphate (pH 5.4), 2-naphthyl α-L-fucopyranoside, 2-naphthyl α-D-glucopyranoside, 2-naphthyl β-D-galactopyranoside, 6-bromo-2-naphthyl α-D-galactopyranoside, 6-bromo-2-naphthyl α-D-mannopyranoside, L-cystyl 2-naphthylamide, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, *N*-benzoyl-DL-arginine 2-naphthylamide and *N*-glutaryl-phenylalanine 2-naphthylamide. Additional physiological characteristics and detailed fatty acid profiles are presented in Tables 1 and 2, respectively. In addition to the data indicated in Table 1, positive for assimilation of 3-hydroxybutyric acid and salicin; negative for acid production from D-glucose and assimilation of sodium acetate, phenylacetic acid, adipic acid, capric acid, trisodium citrate, 3-hydroxybenzoic acid, 4-hydroxybutyric acid, L-fucose, glycogen, inositol, itaconic acid, D-melibiose, D-ribose, potassium 2-ketogluconate, potassium 5-ketogluconate, potassium gluconate, suberic acid and valeric acid. The G+C content of genomic DNA of the type strain is 62.6 mol% (as determined by HPLC).

The type strain, HU1-AH51^T (=LMG 24320^T =KCTC 22311^T), was isolated from freshwater sediment of Lake Hakha, South Korea.

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