**Novosphingobium aquaticum** sp. nov., isolated from the humic-matter-rich bog lake Grosse Fuchskuhle

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A yellow-pigmented, Gram-negative rod, designated FNE08-86T, was isolated from subsurface water of the humic-matter-rich and almost-neutral north-east basin of the experimentally divided bog lake Grosse Fuchskuhle (Brandenburg, Germany). Analysis of the nearly full-length 16S rRNA gene sequence showed the highest 16S rRNA gene sequence similarity with *Novosphingobium rosa* IAM 14222T (96.3%). Sequence similarities with all other members of the genus *Novosphingobium* species were <96%, but phylogenetic tree construction clearly showed the placement of strain FNE08-86T within the genus *Novosphingobium*. The predominant fatty acids were C18:1ω7c and C18:0, and only a single 2-hydroxy fatty acid, C14:0 2-OH, was detected. The polar lipid profile revealed phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine as major compounds, with smaller amounts of sphyngoglycolipid, phosphatidylmonomethylethanolamine, diphosphatidylglycerol and several unidentified lipids. In the quinone system ubiquinone Q-10 was predominant and in the polyamine pattern spermidine was predominant. Characterization by genotypic, chemotaxonomic and phenotypic analysis indicated that strain FNE08-86T represents a novel species of the genus *Novosphingobium*, for which we propose the name *Novosphingobium aquaticum* sp. nov. (type strain FNE08-86T = DSM 25088T = CCM 7983T).

The genus *Novosphingobium* was proposed by Takeuchi et al. (2001) by dissecting the genus *Sphingomonas* based on phylogenetic and chemotaxonomic analysis. At the time of writing, the genus contains 23 species: *Novosphingobium capsulatum* (type species), *N. aromaticivorans*, *N. stygium*, *N. subterraneum*, *N. rosa*, *N. subarcticum* (Takeuchi et al., 2001), *N. resinovorum* (Lim et al., 2007), *N. hassiacum* (Kämpfer et al., 2002), *N. tarda* (Fujii et al., 2003), *N. pentaromativorans* (Sohn et al., 2004), *N. lentum* (Tiirola et al., 2005), *N. taihuense* (Liu et al., 2005), *N. nitrogenifigens* (Addison et al., 2007), *N. naphthalenivorans* (Suzuki & Hiraishi, 2007), *N. acidiphilum* (Glaeser et al., 2009), *N. indicum* (Yuan et al., 2009), *N. mathurensis* and *N. panipatense* (Gupta et al., 2009), *N. sediminicola* (Baek et al., 2011), *N. soli* (Kämpfer et al., 2011), *N. fuchskuhlense* (Glaeser et al., 2012), *N. barchaimii* (Niharika et al., 2012) and *N. lindaniclasticum* (Saxena et al., 2012). Most of these species were isolated from subsurface sediments (Balkwill et al., 1997; Sohn et al., 2004; Baek et al., 2011), soils, often contaminated with polyaromatic compounds (Suzuki & Hiraishi, 2007; Gupta et al., 2009; Kämpfer et al., 2011; Saxena et al., 2012), wastewater treatment plants (Kämpfer et al., 2002; Fujii et al., 2003; Addison et al., 2007), groundwater (Tiirola et al., 2005) and deep seawater (Yuan et al., 2009). *N. acidiphilum* and *N. fuchskuhlense* were recently isolated from two contrasting basins of the experimentally divided, small acidic bog lake Grosse Fuchskuhle in north-eastern Germany (53°10’ N 13°02’ E) (Glaeser et al., 2009, 2012). During field studies on the...
surface water layer of the north-east basin of Grosse Fuchskuhle on 20 June 2008, several more strains were isolated using the dilution-to-extinction method (Button, 1993). All strains were affiliated with the family Sphingomonadaceae, as indicated by 16S rRNA gene sequence analysis. Among those strains, strain FNE08-86T was identified as a potentially novel species of the genus Novosphingobium.

The dilution-to-extinction cultivation conditions have been previously described in detail (Glaeser et al., 2012). Subcultivation and maintenance of strain FNE08-86T was performed in liquid medium or on agar using K7 medium (0.1% each yeast extract, peptone and glucose) adjusted to pH 6.5 at 25 °C. For long-term storage at −280 °C, 6% DMSO or 20% glycerol were added to liquid cultures.

For phylogenetic analysis, DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Glaeser et al., 2012). Phylogenetic trees were calculated in ARB release 5.2 (Ludwig et al., 2004) using the All-Species Living Tree Project (Yarza et al., 2008) database (release LTPs106, August 2011). SINA version 1.2.9 was used to align sequences. The alignment was corrected manually with respect to the secondary structure information. Phylogenetic trees were calculated with the maximum-likelihood method using RAxML version 7.04 (Stamatakis, 2006) and the GTR-GAMMA model and PhyML with the substitution model HKY85 (Hasegawa et al., 1985), the neighbour-joining method with the Jukes–Cantor correction (Jukes & Cantor, 1969) and the maximum-parsimony method with DNAPARS version 3.6 (Felsenstein, 2005). For all analyses, bootstrap values (>70%) based on 100 resamplings are shown at branch nodes. Single asterisks indicate that the corresponding nodes were recovered in trees generated with the maximum-likelihood (PhyML) and neighbour-joining method. Double asterisks indicate that the corresponding nodes were recovered in trees generated with the maximum-likelihood (PhyML and RAxML) and, neighbour-joining method. Sphingosinicella microcystinivorans Y2T was used as an outgroup. Bar, 0.10 substitutions per site.
isolated from the same habitat and other members of the genus "Novosphingobium".

The sequenced 16S rRNA gene fragment of strain FNE08-86T represented a continuous stretch of 1355 unambiguous nucleotides characteristic for the genus "Novosphingobium" as reported by Takeuchi et al. (2001) were present in the 16S rRNA gene sequence of strain FNE08-86T, as for all other "Novosphingobium" species.

Colony morphology was examined on K7 agar (pH 6.5) after 72 h of incubation at 25°C. Cell morphology and motility were subsequently investigated on agar-covered plates. The 16S rRNA gene sequence of strain FNE08-86T clustered with the type strain of IAM 14222T and N. sediminicola HU1-AH51T (Fig. 1). The nodes were not supported with high bootstrap values and were not obtained in trees generated with the maximum-likelihood method using RAxML. All of the 16S rRNA gene signature nucleotides characteristic for the genus "Novosphingobium" were present in the 16S rRNA sequences of the two "Novosphingobium species" also isolated from the same habitat as reported by Takeuchi et al. (2001) were present in the 16S rRNA gene sequence of strain FNE08-86T, as for all other "Novosphingobium" species.

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slides by phase-contrast microscopy at ×1000 magnification using an Eclipse 80i microscope (Nikon Europe, Badhoevedorp, Netherlands). Gram-staining was performed by the modified Hucker method according to Gerhardt et al. (1994). Cytochrome c oxidase activity was tested using the Bactident oxidase test (Merck), and catalase activity was tested by gas formation after addition of 3 % H2O2. The formation of capsules was determined after staining with China ink. Cell morphology was investigated in further detail by transmission electron microscopy as described previously (Glaeser et al., 2012). Transmission electron micrographs indicated Gram-negative cell walls without any intracytoplasmic membrane systems (Fig. 2). Small dense granules were distributed all over the cytoplasm, as observed for N. fuchskuhlense previously (Glaeser et al., 2012).

Detailed phenotypic characterization of strain FNE08-86T was performed in comparison with N. rosa NBRC 15208T, N. fuchskuhlense FNE08-7T, N. stygium SMCC B0712T, N. taihuenense DSM 17507T, N. acidiphilum FSW06-204dT and N. capsulatum DSM 30196T. Growth at 0.5, 0.75 and 1.0 % (w/v) NaCl and at pH 4.0–9.0 (at intervals of 0.5 pH units) was investigated on K7 medium. Substrate utilization was considered positive when the OD600 of the substrate-amended cultures was increased by >0.05 OD units in comparison to control cultures grown without addition of a carbon substrate. Strain FNE08-86T could be clearly distinguished from other members of the genus Novosphingobium by several features (Table 1). The detailed phenotypic characterization of strain FNE08-86T is given in the species description.

Fatty acids were analysed as described by Kämpfer & Kroppenstedt (1996) using cell biomass grown for 72 h on K7 agar at pH 6.5 (late exponential growth phase). The predominant fatty acid of strain FNE08-86T was C18:1ω7c (54.9 %), followed by C16:0 (20 %). Only one 2-hydroxy fatty acid, C14:0 2-OH (8.2 %), was detected. The fatty acid composition was characteristic for species of the genus Novosphingobium (Table 2).

Quinones and polar lipids were extracted by the integrated procedure (Tindall, 1990a, b; Altenburger et al., 1996). Polyamines were extracted as reported by Busse & Auling (1988). Quinones and polyamines were analysed by HPLC according to Stolz et al. (2007). Polar lipids were analysed by two-dimensional TLC applying molybdophosphoric acid for detection of total lipids, ninhydrin for detection of aminolipids, molybdenum blue for detection of phospholipids, α-naphthol for detection of glycolipids and Dragendorff’s reagent for detection of phosphatidyglycerol (Tindall, 1990b). The quinone system of strain FNE08-86T was composed of ubiquinone Q-10 (85 %), Q-9 (10 %) and Q-11 (5 %). The polyamine pattern consisted of the major compound spermidine [42.3 μmol (g dry weight)−1] and traces [<0.5 μmol (g dry weight)−1] of putrescine and cadaverine. The polar lipid profile (Fig. 3) showed the major lipids phosphatidylethanolamine, phosphatidylglycerol and phosphatidylycholine. Additionally, the following lipids were present in lower amounts: sphingoglycolipid, phosphatidylmonomethylethanolamine, diphosphatidylglycerol, phosphatidylcholine, phosphatidylcholine, and phosphatidylethanolamine.

Table 2. Whole-cell fatty acid compositions (%) of strain FNE08-86T, its closest phylogenetic neighbour, the two Novosphingobium species isolated from the same habitat and other members of the genus Novosphingobium

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4*</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td></td>
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</tr>
<tr>
<td>C14:0</td>
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<td>−</td>
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<td>1.5</td>
<td>1.8</td>
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<td>−</td>
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<tr>
<td>C15:0</td>
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<td>3.4</td>
<td>6.1</td>
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<tr>
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<td>7.1</td>
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<td>16.4</td>
<td>11.7</td>
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<td>−</td>
<td>−</td>
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<tr>
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<td>7.3</td>
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*K7 medium was adjusted to pH 4.7.
†Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C16:1ω7c and/or iso-C15:0 2-OH.
glycerol, one phospholipid, one aminolipid, two glycolipids showing highly hydrophilic chromatographic motility and four lipids not stainable with specific reagents. A fifth lipid (stretched spot) showed a weak reaction with ninhydrin, but it did not demonstrate unambiguously the presence of an amino group. Furthermore, a single yellow pigment with highly hydrophobic motility is present. Presence of a pigment with similar chromatographic motility was also reported for *N. soli* (Kämpfer *et al.*, 2011), *N. acidiphilum* (Glaeser *et al.*, 2009) and *N. fuchskuhlense* (Glaeser *et al.*, 2012). The quinone system, polyamine pattern and polar lipid profile are in perfect agreement with the description of the genus Novosphingobium (Takeuchi *et al.*, 2001). Phosphatidyl(dimethylethanolamine) (PDE), as found in *N. lentum* (Tiirola *et al.*, 2005), *N. acidiphilum* (Glaeser *et al.*, 2009), *N. soli* (Kämpfer *et al.*, 2011), *N. mathurensense* (Gupta *et al.*, 2009), *N. aromativorans*, *N. capsulatum*, *N. rosa*, *N. resinovorum*, *N. stygium* and *N. subterraneum* (Busse *et al.*, 1999), could not be detected. Instead, an unidentified phospholipid and an unidentified aminolipid were detected, demonstrating a chromatographic motility most similar to that expected for phosphatidyl(dimethylethanolamine). These two lipids appear to be useful to distinguish similar to that expected for phosphatidyldimethylethanolamine. These two lipids appear to be useful to distinguish strain FNE08-86T from all of the above-mentioned species lacking these lipids and showing instead the presence of PDE. However, it is possible that the two unidentified lipids exhibit under certain but unknown conditions the identical chromatographic motility resulting after 2D-TLC in a single spot that shows the chromatographic motility and staining behaviour of PDE. Hence, before the presence of the unidentified phospholipid and unidentified aminolipid is considered to be unique within the genus *Novosphingobium* and useful for discrimination, the presence of PDE in the above-mentioned species needs to be carefully reanalysed.

Based on the genotypic, chemotaxonomic and phenotypic characterization, we conclude that strain FNE08-86T represents a novel species of the genus *Novosphingobium*, for which we propose the name *Novosphingobium aquaticum* sp. nov.

**Description of Novosphingobium aquaticum** sp. nov.

*Novosphingobium aquaticum* (aqua’ti.cum. L. neut. adj. aquaticum living in water, aquatic).

Exhibits the characteristics of the genus *Novosphingobium* as summarized by Takeuchi *et al.* (2001). Small, circular, yellow colonies (1–2 mm in diameter) are formed after 2–3 days at 25 °C on K7 agar (pH 6.5). Cells are Gram-negative, non-motile rods (2.0 ± 0.2 μm long and 0.9 ± 0.2 μm wide). Weakly positive for cytochrome c oxidase and positive for catalase. Capsules are formed. Grows at 4 and 32 °C, but not at 37 or 40 °C. Grows well with 0–0.25 % (w/v) NaCl and weakly with 0.25–0.75 % NaCl, but does not grow with 1 % NaCl. Grows well at pH 4.0–6.5 and weakly at pH 6.5–8.0. Grows well on K7, R2A, M402 and M1209 agar and grows weakly on peptone yeast extract, but does not grow on nutrient, tryptic soy, glycine-arginine, CASO, M65, McConkey, LB, marine or blood agar. Assimilates L-(+)-arabinose, cellobiose, D-(−)-fructose, D-(−)-galactose, D-glucose, D-mannose, maltose, sucrose and L-proline, but not L-rhamnose, sorbose, D-xyllose, D-3-amino-butyrat, DL-lactate, L-malate, formate, pyruvate, oxalate, L-histidine, L-leucine or L-phenylalanine. Positive for hydrolysis of pNP-α-D-glucopyranoside, bis-pNP-phosphate, pNP-phenylphosphonate, pNP-phosphorylcholine and L-alanine-pNA, weakly positive for hydrolysis of asescin and L-proline-pNA and negative for the hydrolysis of pNP β-D-galactopyranoside, pNP-β-D-glucuronide, pNP-β-D-glucopyranoside, 2-deoxythymidine-5‘-pNP-phosphate and L-glutamate-γ-3-carboxy-pNA. The major fatty acids are C18:1ω7c and C16:0. Only a single 2-hydroxy fatty acid, C14:0 2-OH, is present. The polar lipid profile consists of major amounts of phosphatidyethanolamine, phosphatidylglycerol and phosphatidylethanolamine and minor amounts of sphingoglycolipid, phosphatidylmonomethylethanolamine, diphasphatidylglycerol, an unidentified phospholipid, an unidentified aminolipid, five unknown glycolipids, five unknown lipids without a phosphate group, an amino group or sugar moiety and a yellow pigment staining with α-naphthol.

**Fig. 3.** Two-dimensional TLC of polar lipid extracts of strain FNE08-86T stained with molybdathophosphoric acid. AL, Unknown aminolipid; DPG, diphasphatidylglycerol; GL, unknown glycolipid; L, unknown lipid not stained with specific staining reagents; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unknown phospholipid; PME, phosphatidylmethylethanolamine; SGL, sphingoglycolipid; yPigm, yellow pigment staining with α-naphthol.
Q-9 and Q-11. The major component in the polyamine pattern is spermidine.

The type strain, FNE08-86T (=DSM 25088T=CCM 7983T), was isolated from a subsurface water sample of the north-east basin of the bog lake Grosse Fuchskuhle, north-eastern Germany, which is humic-matter-rich and has an almost-neutral pH.

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References


