Novosphingobium acidiphilum sp. nov., an acidophilic salt-sensitive bacterium isolated from the humic acid-rich Lake Grosse Fuchskuhle

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A yellow- to orange-pigmented, Gram-negative, rod-shaped, motile and non-spore-forming bacterium, strain FSW06-204d^T, was isolated from subsurface water of the acidic bog lake, Lake Grosse Fuchskuhle (Brandenburg, Germany). Optimum growth of this strain occurred over a pH range from 5.5 to 6.0 and the growth rate strongly decreased at pH values above 6.5. In addition, the strain exhibited a low tolerance towards NaCl and grew only at a NaCl concentration of up to 0.5 %. 16S rRNA gene sequence analysis of strain FSW06-204d^T showed the highest sequence similarity to *Novosphingobium hassiacum* W-51^T (96.7 %) and formed a distinct cluster with *Novosphingobium nitrogenifigens* DSM 19370^T (96.4 %) within the genus *Novosphingobium*. Strain FSW06-204d^T shared a 21 bp signature gap with the latter species, a feature that is absent in all other members of the family *Sphingomonadaceae*. DNA–DNA hybridization of strain FSW06-204d^T and *N. nitrogenifigens* DSM 19370^T showed a low relatedness value of 24 % (reciprocal: 39 %). The major respiratory quinone was ubiquinone Q-10 (91 %) and the predominant fatty acid was C_{18:1}ω7c (43.3 %). Two characteristic 2-hydroxy fatty acids, C_{14:0} 2-OH (8.1 %) and C_{15:0} 2-OH (6.5 %), were abundant. Polar lipids consisted mainly of phosphatidylmethylethanolamine and phosphatidylethanolamine; however, only moderate amounts of sphingoglycolipids were present and phosphatidylcholine was lacking. Characterization by 16S rRNA gene sequence, physiological features, pigment analysis and polyamine, ubiquinone, polar lipid and fatty acid contents revealed that strain FSW06-204d^T represents a novel species of the genus *Novosphingobium* within the class *Alphaproteobacteria*. The name *Novosphingobium acidiphilum* sp. nov. is proposed for this acidophilic and salt-sensitive species with the type strain FSW06-204d^T (=DSM 19966^T=CCM 7496^T=CCUG 55538^T).

The genus *Novosphingobium* was proposed as a result of the dissection of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990) based on phylogenetic and chemotaxonomic analysis

Abbreviations: pNA, para-nitroanilide; pNP, para-nitrophenyl.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain FSW06-204d^T is EU336977.

The results of a comparative pigment analysis for strain FSW06-204d^T and related strains are presented in a supplementary table available with the online version of this paper. Supplementary figures showing a maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences, the polar lipid profile of strain FSW06-204d^T and the results of a pigment analysis for the novel strain are also available.

with *Novosphingobium capsulatum* as the type species (Takeuchi *et al.*, 2001). The genus *Novosphingobium* currently comprises 14 recognized species. Members of the genus *Novosphingobium* are rather heterogeneous and originate from a variety of habitats including soil, coastal or freshwater sediments (Balkwill *et al.*, 1997; Sohn *et al.*, 2004; Liu *et al.*, 2005; Suzuki & Hiraishi 2007), activated sludge/wastewater treatment plants (Neef *et al.*, 1999; Fujii *et al.*, 2003) and a contaminated groundwater bioremediation reactor (Tirola *et al.*, 2002). In 16S rRNA gene clone libraries of subsurface water samples of the acidic and humic acid-rich Lake Grosse Fuchskuhle (Mecklenburg-Brandenburg lake district, north-eastern Germany), a 16S

rRNA gene sequence frequently appeared that was affiliated to the genus *Novosphingobium* (Glöckner *et al.*, 2000; Burkert *et al.*, 2003; Allgaier & Grossart, 2006). In contrast to most of the recognized species of this genus, 21 bp were lacking in the 16S rRNA gene sequence of this uncultured *Novosphingobium* species. The same 21 bp gap has recently been found in the 16S rRNA gene sequence of *Novosphingobium nitrogenifigens* isolated from a paper mill in New Zealand (Addison *et al.*, 2007).

During field studies in July 2006, a yellow- to orange-pigmented bacterium (strain FSW06-204d^T) was isolated from subsurface water samples of the south-west (SW) basin of Lake Grosse Fuchskuhle. At the time of isolation, the pH of the SW basin was 4.7. The 16S rRNA gene sequence of strain FSW06-204d^T showed 99% similarity to the cloned 16S rRNA gene sequences obtained from the SW basin of Lake Grosse Fuchskuhle (Glöckner *et al.*, 2000; Burkert *et al.*, 2003; Allgaier & Grossart, 2006). Strain FSW06-204d^T was enriched in a most-probable-number (MPN) dilution series of water samples performed in microtitre plates using synthetic freshwater (Bartscht *et al.*, 1999) containing 10 µM NH₄Cl, 10 µM KH₂PO₄, 100 µM KNO₃, 200 µM MgSO₄·7H₂O, 100 µM CaCl₂·2H₂O and 250 µM CaCO₃, 1 ml l⁻¹ of SL 10 trace elements (Tschech & Pfennig, 1984) and 10 ml l⁻¹ vitamin solution (Balch *et al.*, 1979) buffered with 10 mM HEPES (pH 7.2) and supplemented with 0.001% (v/v) Tween 20, a mixture of six amino acids (L-cysteine, L-alanine, L-isoleucine, L-leucine, L-asparagine and L-arginine, each 200 µM), a fatty acid mixture of formate, acetate and propionate (each 200 µM), as well as with glucose, pyruvate, citrate, 2-oxoglutarate and succinate (each 200 µM) (Jaspers *et al.*, 2001). After 8 weeks incubation at room temperature in the dark, the novel strain was isolated from one of the highest positive dilution steps by streaking subsamples on YPG agar plates [1.2% agar supplemented with a 0.22 µm filter-sterilized concentrate of yeast extract, peptone and glucose with final concentrations of 0.075, 0.15 and 0.075% (w/v), respectively]. After 4 weeks incubation at room temperature in the dark, orange-pigmented colonies were visible. Purification of single colonies was performed in YPG top agar dilution series (0.8% agar) and the strain was preserved at -80 °C in 7% DMSO.

Strain FSW06-204d^T formed small yellow colonies (<0.5 mm) with a smooth surface after 48 h at 25 °C on YPG agar plates. Colonies turned orange after a few more days of incubation. Cell morphology was investigated by phase-contrast microscopy (Fig. 1a) for cells grown in K7 medium (0.1% yeast extract, peptone and glucose) adjusted to pH 5.5 at 25 °C. During exponential growth, the rod-shaped cells of strain FSW06-204d^T were 1.4±0.4 µm long and 0.6±0.1 µm wide. Cells were slightly elongated in the stationary phase (1.6±0.3 and 0.6±0.1 µm), motile in the early exponential phase and of shorter appearance when grown in K7 medium without glucose. Electron microscopy of ultrathin cell sections stained with 1% uranyl acetate showed a typical Gram-

Fig. 1. Phase-contrast micrographs of exponentially growing cells of strain FSW06-204d^T (a) and electron micrographs showing ultrathin sections of exponentially growing cells of strain FSW06-204d^T obtained by contrasting with uranyl acetate (b, c). OM, outer membrane; CM, cytoplasmic membrane; IG, intracellular granules. Bars, 5 µm (a), 0.5 µm (b, c).

negative cell wall and no intracytoplasmic membrane systems (Fig. 1b and c). In some cells, single electron-dense polar granules were observed which were similar to those observed for *Sandarakinorhabdus limnophila* so42^T, also isolated from a freshwater lake (Gich & Overmann, 2006). Polyhydroxyalkanoate granules were not observed in cells of the novel strain in contrast to observations of *N. nitrogenifigens* DSM 19370^T (Addison *et al.*, 2007). Cells of strain FSW06-204d^T grown on YPG agar plates adjusted to pH 5.5 stained Gram-negative and were positive for cytochrome oxidase in a delayed reaction as determined by using an oxidase test (Merck). Since gas was not produced after the addition of 3% H₂O₂, strain FSW06-

204d^T was deemed to be catalase-negative. Endospores were not detected after staining with malachite green and capsules were lacking as determined by contrasting a bacterial suspension with china ink. Obligate aerobic to microaerophilic growth was indicated by growth at the surface and in a zone approximately 1 cm below the surface of YPG soft agar tubes (0.16 % agar, pH 5.5).

Temperature, salinity and pH-dependent growth were examined in K7 medium buffered with 10 mM filter-sterilized MES. The pH values of the K7 medium and MES buffer were adjusted using KOH and HCl. Growth experiments were performed in a total volume of 6 ml in 25 ml screw cap tubes under continuous shaking at 220 r.p.m. resulting in an oxygen concentration of 4–6 mg l⁻¹ as measured with an oxygen sensor (OxyScan Oxygen sensor 501, Aquatech). Optical density was monitored at 600 nm and all experiments were performed in duplicate and repeated twice. In MES-buffered K7 medium, growth occurred between pH 4.5 and 7.0, but not at pH 3.5 or 7.5. Highest growth rates were observed at pH 5.5 and 6.0. On YPG agar plates, growth was observed at between 4 and 32 °C, but not at 37 or 42 °C. In liquid medium, a minimum doubling time of 5.3 h was determined at 25 °C. Salinity-dependent growth was examined by the addition of NaCl (w/v) in 0.25 % (w/v) steps. Highest growth rates were observed when no or 0.25 % (w/v) NaCl were added, but decreased in the presence of 0.5 % (w/v) NaCl and growth was not observed after the addition of 0.75 % (w/v) NaCl. In summary, the optimal growth conditions for strain FSW06-204d^T were pH 5.5 to 6.0, 25 °C and with no addition of NaCl.

For phylogenetic characterization, chromosomal DNA was extracted as described by Pitcher *et al.* (1989) and a 1376 bp fragment of the 16S rRNA gene was amplified using primers targeting conserved regions of the gene, 8F (8–27, *Escherichia coli* numbering, Brosius *et al.*, 1981) and 1492R (1492–1510) (Lane, 1991). Sequencing of the DNA fragment was performed with primers 8F, 1492R and two additional reverse primers, 907R (907–926) and 1100R (1100–1115) (Lane, 1991), using the Big Dye terminator cycle sequencing reaction kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems). DNA sequences were processed in MEGA (molecular evolutionary genetics analysis) version 3 software (Kumar *et al.*, 2004) and phylogenetic analysis was performed with ARB software (Ludwig *et al.*, 2004). A pairwise distance matrix was calculated and phylogenetic trees were constructed using the neighbour-joining and maximum-parsimony methods including bootstrap values based on 1000 resampled datasets. Because strain FSW06-204d^T lacked a 21 bp sequence between positions 1256 and 1278 (*E. coli* numbering, Brosius *et al.*, 1981), the respective 21 bp were manually removed from the sequences of the reference strains included in the calculations. Similarity values and phylogenetic trees were calculated with gaps included and after excluding gap positions.

A comparison of the 16S rRNA gene sequences of strain FSW06-204d^T and the type strains of all the recognized species of the genus *Novosphingobium* revealed that the novel strain showed the highest similarity to *Novosphingobium hassiacum* (GenBank accession no. AJ416411, 96.7 %), followed by *N. capsulatum* (D16147, 96.6 %), *Novosphingobium taihuense* (AY500142, 96.5 %) and *N. nitrogenifigens* (DQ448852, 96.4 %). The 16S rRNA gene sequence similarity to other species of the genus *Novosphingobium* was in a range from 94.2 to 96.4 %. All signature nucleotides characteristic for the genus *Novosphingobium* as reported by Takeuchi *et al.* (2001) were present in the 16S rRNA gene sequence of strain FSW06-204d^T. A 21 bp gap already described for *N. nitrogenifigens* (Addison *et al.*, 2007) was present in the 16S rRNA gene sequence of strain FSW06-204d^T between base positions 1256 and 1278 (*E. coli* numbering, Brosius *et al.*, 1981). This 21 bp gap was not present in other recognized species of the family *Sphingomonadaceae* but occurred frequently in other members of the class *Alphaproteobacteria*, for example, in *Filomicrobium fusiforme* DSM 5304^T and the closely related *Rhodobium marinum* DSM 2698^T, but not in other closely related species (Rainey *et al.*, 1998). In contrast, the gap seemed to be a common feature only within the family *Rhodobacteraceae*, as observed by searching within the ARB database (Ludwig *et al.*, 2004). The presence of this 21 bp signature gap distinguished strain FSW06-204d^T and *N. nitrogenifigens* DSM 19370^T from other species of the genus *Novosphingobium*. These two strains formed a distinct cluster within the currently recognized members of the genus *Novosphingobium* as shown by the calculation of phylogenetic trees when the gap region was excluded from the analysis (Fig. 2 and Supplementary Fig S1 available in IJSEM Online). When the region of the 21 bp gap was included in the same calculations, no differences were revealed in the phylogenetic relationships of strain FSW06-204d^T. The gene sequence similarity values of strain FSW06-204d^T to other *Novosphingobium* species were only slightly different in this analysis. For *N. hassiacum* (GenBank accession no. AJ416411), similarity values were 97.2 % instead of 96.7 % and for *Novosphingobium stygium* (U20775), 95.6 % instead of 95.5 %. Therefore, the cluster formed by strains FSW06-204d^T and *N. nitrogenifigens* DSM 19370^T was independent of the 21 bp gap.

DNA–DNA hybridization experiments with strains FSW06-204d^T and *N. nitrogenifigens* DSM 19370^T were performed as described previously (Kämpfer *et al.*, 2002) and revealed a low DNA–DNA relatedness between the strains. Cross-hybridization of genomic DNA from FSW06-204d^T to *N. nitrogenifigens* DSM 19370^T yielded a relatedness value of 39 %, whereas the reciprocal hybridization gave a lower value of 24 %.

Further characterization of strain FSW06-204d^T was first performed using a substrate assimilation panel and enzyme tests with chromogenic substrates [p-nitrophenyl- (pNP)

Fig. 2. Phylogenetic tree calculated using the neighbour-joining method indicating the phylogenetic relationship of strain FSW06-204d^T to other species of the genus *Novosphingobium*. For phylogenetic analysis, sequences containing 1375 bp of the 16S rRNA gene were aligned excluding the region of the 21 bp gap present in strain FSW06-204d^T (1256–1278, *E. coli* numbering, Brosius *et al.*, 1981). *Erythrobacter longus* ATCC 33941^T was used as the outgroup. Numbers at nodes indicate the percentage levels of bootstrap support based on 1000 resampled datasets. Bar, 1 substitution per 100 nucleotides.

and p-nitroanilide- (pNA) linked substrates] (Kämpfer *et al.*, 1991). In contrast to the inoculation procedure described by Kämpfer *et al.* (1991), strain FSW06-204d^T was not inoculated after resuspension in 0.9% (w/v) NaCl because of its sensitivity to salt. Instead, a culture of strain FSW06-204d^T was resuspended in MES buffer (pH 5.5) to give a final concentration of 50 mM MES for incubation. The pH of the phosphate buffer was adjusted to the pH range for optimum growth for FSW06-204d^T. Microtitre plates were incubated for 7 to 14 days at room temperature in the dark. In contrast to all other species of the genus *Novosphingobium* examined using these tests (Table 1, data from Kämpfer *et al.*, 2002; Tiirila *et al.*, 2005), growth of strain FSW06-204d^T and also of *N. nitrogenifigens* DSM 19370^T was only observed in the media containing the chromophoric substrates and was not observed in the substrate assimilation media (Kämpfer *et al.*, 1991). Therefore substrate assimilation tests for strains FSW06-204d^T, *N. nitrogenifigens* DSM 19370^T and the closest related type strain, *N. hassiacum* W-51^T, were performed with a selection of substrates in twofold diluted, glucose-free K7 medium with 10 mM MES (pH 5.5). Substrates were added to a final concentration of 5 mM. Tests were performed in duplicate using two independent pre-cultures for inoculation. Substrate utilization was considered positive if the OD₆₀₀ value of the substrate-amended cultures increased by more than 0.05 OD units in comparison with the control cultures grown in parallel without the addition of a carbon substrate. The results for the substrate assimilation tests as compared with those from the literature for the recognized species of the genus *Novosphingobium* are presented in Table 1. The fact that strains FSW06-204d^T and *N. nitrogenifigens* DSM 19370^T were not able to grow on the assimilation medium meant that these strains could be differentiated from other species of the genus *Novosphingobium* on the basis of their growth requirements. The results of the substrate assimilation tests for *N. hassiacum* W-51^T (Table 1) were identical overall when the tests were performed with either our method or with the method of Kämpfer *et al.* (1991). Our results show that strain FSW06-204d^T can be distinguished from *N.*

nitrogenifigens DSM 19370^T and *N. hassiacum* W-51^T by 12 and 14 traits, respectively, of 31 tested characteristics listed in Table 1. Strain FSW06-204d^T can be separated from all other species of the genus *Novosphingobium* by several characteristics when compared with the data from the literature (Table 1).

Fatty acids were analysed from biomass that was grown for 72 h on K7 agar plates at 22 °C (strain FSW06-204d^T and *N. hassiacum* W-51^T) and 28 °C (*N. nitrogenifigens* DSM 19370^T) as described by Kämpfer & Kroppenstedt (1996). The predominant fatty acids of strain FSW06-204d^T were C_{18:1}ω7c (43.3%) and C_{17:1}ω6c (17.6%). Two major 2-hydroxy fatty acids, C_{14:0} 2-OH (8.1%) and C_{15:0} 2-OH (6.5%), were also present, but no 3-hydroxy fatty acids were detected (Table 2). The genus *Novosphingobium* was distinguished from the genera *Sphingomonas sensu stricto*, *Sphingopyxis* and *Sphingobium* by the absence of other major 2-hydroxy fatty acids in addition to C_{14:0} 2-OH (Takeuchi *et al.*, 2001). However, strain FSW06-204d^T contained rather similar amounts of fatty acids C_{14:0} 2-OH and C_{15:0} 2-OH. This profile has also been shown for *N. hassiacum* W-51^T (Kämpfer *et al.*, 2002). The detailed fatty acid profile for strain FSW06-204d^T is shown in Table 2 and shows the affiliation of strain FSW06-204d^T to the genus *Novosphingobium* (Takeuchi *et al.*, 2001) and the differences observed between this strain and the closest related type strains, *N. hassiacum* W-51^T and *N. nitrogenifigens* DSM 19370^T.

Biomass for polyamine, quinone and polar lipid analyses was grown in K7 medium adjusted to pH 4.5. Polyamines were analysed as described by Busse & Auling (1988) and Stolz *et al.* (2007). Respiratory quinones and polar lipids were determined according to Tindall (1990a, b) and Altenburger *et al.* (1996). The polyamine pattern of strain FSW06-204d^T, with the predominant compound spermidine (31.8 μmol g⁻¹ dry weight) and small amounts of putrescine (0.1 μmol g⁻¹ dry weight) and spermine (0.2 μmol g⁻¹ dry weight), was in accordance with those of all other species of the genus *Novosphingobium* so far examined for this trait (Busse *et al.*, 1999; Takeuchi *et al.*,

Table 1. Characteristics that differentiate strain FSW06-204d^T from recognized species of the genus *Novosphingobium*

Strains: 1, FSW06-204d^T; 2, *N. nitrogenifigens* DSM 19370^T; 3, *N. hassiacum* W-51^T; 4, *N. lentum* MT1^T; 5, *N. tardaugs* JCM 11434^T; 6, *N. rosa* IFO 15208^T; 7, *N. capsulatum* ATCC 14666^T; 8, *N. stygium* SMCC B0712^T; 9, *N. subterraneum* SMCC B0478^T; 10, *N. aromaticivorans* SMCC F199^T; 11, *N. subarcticum* HAMBI 2110^T; 12, *N. pentaromaticivorans* KCTC 10454^T; 13, *N. taihuense* T3-B9^T; 14, *N. naphthalenivorans* TUT562^T. Data for strains 6–11 were taken from Kämpfer *et al.* (2002), for strains 4, 5, and 10 from Tirola *et al.* (2005), for strain 13 from Liu *et al.* (2005) and for strain 14 from Suzuki & Hiraishi (2007). –, Negative; +, positive; (+), weakly positive; pNA, para-nitroanilide; pNP, para-nitrophenyl; ND, not detected or not described. All strains were negative for the assimilation of D-mannitol and citrate.

Characteristic	1*	2*	3*	3†	4†	5†	6†	7†	8†	9†	10†	11†	12†	13‡	14‡
Hydrolysis of:															
Aesculin	+	–	–	–	–	–	(+)	–	+	+	+	+	+	+	+
pNP-β-D-galactopyranoside	–	–	–	–	–	–	+	+	–	(+)	+	–	–	ND	ND
pNP-β-D-glucuronide	–	–	–	–	–	–	+	+	–	+	+	+	+	ND	ND
pNP-α-D-glucopyranoside	–	+	+	+	+	–	+	+	+	+	+	+	+	ND	ND
pNP-β-D-glucopyranoside	–	–	–	–	–	–	+	+	+	+	+	+	+	ND	ND
bis-pNP-phosphate	–	–	+	+	–	+	+	+	(+)	(+)	+	+	+	ND	ND
pNP-phenylphosphonate	–	–	+	+	–	+	+	+	–	+	+	+	–	ND	ND
pNP-phosphorylcholine	–	–	–	–	–	(+)	+	+	–	–	(+)	–	+	ND	ND
2-Deoxythymidine-5'-pNP-phosphate	–	+	+	+	–	–	+	+	+	+	+	+	+	ND	ND
L-Alanine-pNA	+	+	+	+	+	(+)	+	+	(+)	+	+	+	+	ND	ND
L-Glutamate-γ-3-carboxy-pNA	+	–	+	+	–	–	–	+	–	+	+	+	–	ND	ND
L-Proline-pNA	+	–	–	–	–	–	–	–	–	–	+	–	–	ND	ND
Assimilation of:															
L-Arabinose	(+)	(+)	–	–	–	–	+	(+)	–	(+)	–	+	–	+	+
Cellobiose	+	+	+	+	–	–	+	+	–	+	+	–	–	+	ND
D-Fructose	(+)	+	–	–	–	–	+	–	–	–	–	–	+	+	ND
D-Galactose	+	–	–	–	–	–	–	+	–	–	–	+	–	–	ND
D-Glucose	+	+	+	+	–	–	+	+	–	+	+	+	–	+	+
D-Mannose	+	+	(+)	–	–	–	+	(+)	–	+	+	–	–	+	–
Maltose	+	+	–	–	–	–	+	+	–	+	+	+	–	+	+
L-Rhamnose	+	–	–	–	–	–	+	+	–	+	+	+	+	+	ND
Sucrose	+	–	+	+	–	–	+	+	–	+	+	–	+	+	ND
D-Xylose	–	–	–	–	–	–	+	+	–	+	+	+	–	+	ND
DL-Lactate	–	+	–	–	–	+	–	–	–	–	–	+	–	ND	ND
L-Malate	+	+	–	–	–	–	+	+	–	–	–	+	–	ND	–
2-Oxoglutarate	+	–	–	–	–	–	–	–	–	–	–	–	–	ND	ND
L-Histidine	–	+	–	–	–	–	+	+	–	–	–	+	–	ND	ND
L-Leucine	(+)	–	–	–	–	–	–	+	–	–	+	+	–	ND	ND
L-Phenylalanine	–	–	–	–	–	–	–	–	–	–	–	–	+	–	ND
L-Proline	+	+	+	+	–	–	–	–	–	+	+	+	+	+	ND

*Hydrolysis tests were performed by the method of Kämpfer *et al.* (1991) and substrate assimilation tests by the method optimized for strain FSW06-204d^T.

†Hydrolysis and substrate assimilation tests were performed according to the method of Kämpfer *et al.* (1991).

‡Substrate assimilation tests were performed with methods described by Liu *et al.* (2005) and Suzuki & Hiraishi (2007).

2001; Kämpfer *et al.*, 2002; Tirola *et al.*, 2005). Strain FSW06-204d^T exhibited a quinone system consisting of ubiquinone Q-10 (91%) and Q-9 (9%), which was in accordance with the characteristics of the genus (Busse *et al.*, 1999; Takeuchi *et al.*, 2001; Kämpfer *et al.*, 2002; Tirola *et al.*, 2005). The polar lipid profile consisted of the major compounds phosphatidylmethylethanolamine and phosphatidylethanolamine, moderate amounts of a highly

hydrophobic yellow pigment stainable with α-naphthol, moderate amounts of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylglycerol, sphingoglycolipid, an unknown highly hydrophilic amino-phosphoglycolipid, two unknown polar lipids and minor to trace amounts of one unknown glycolipid, two unknown phospholipids, two unknown polar lipids and another highly hydrophobic yellow pigment (see Supplementary

Table 2. Whole-cell fatty acid profiles of strain FSW06-204d^T, *N. hassiacum* W-51^T and *N. nitrogenifigens* DSM 19370^T

Strains: 1, FSW06-204d^T; 2, *N. hassiacum* W-51^T; 3, *N. nitrogenifigens* DSM 19370^T. Values represent the percentage of total fatty acids. Localization of double bonds is given by counting from the methyl (ω) end of the carbon chain. Summed features 2 and 3 represent groups of two or three fatty acids that could not be separated by GLC in the MIDI system. Summed feature 2 contains C_{14:0} 3-OH/C_{16:1} iso I and summed feature 3 contains C_{16:1} ω 7c/C_{15:0} iso 2-OH.

Fatty acids	1	2	3
Saturated fatty acids:			
C _{14:0}	1.5	—	1.0
C _{15:0}	6.1	1.2	—
C _{16:0}	8.7	7.3	6.3
C _{17:0}	3.8	—	—
C _{18:0}	—	—	1.0
Unsaturated fatty acids:			
C _{17:1} ω 8c	—	1.1	—
C _{17:1} ω 6c	17.6	8.9	1.2
C _{18:1} ω 7c	43.3	30.7	61.5
11 methyl C _{18:1} ω 7c	—	4.0	—
Hydroxy fatty acids:			
C _{14:0} 2-OH	8.1	17.9	16.1
C _{15:0} 2-OH	6.5	5.5	—
Summed feature 2	—	—	0.2
Summed feature 3	3.5	23.3	12.8

Fig. S2 in IJSEM Online). Overall, this profile showed a high similarity to those of other *Novosphingobium* species but characteristic features of strain FSW06-204d^T were the lack of phosphatidylcholine and a moderate content of sphingoglycolipids.

Pigment analysis was performed with cells of strain FSW06-204d^T that were in the exponential growth phase in K7 medium adjusted to pH 5.5 at 25 °C. Absorbance spectra of acetone/methanol (7/2, v/v) extracts were characterized by $\lambda_{\max}$ at 453 and 480 nm and cell-free extracts were characterized by $\lambda_{\max}$ at 432/433, 458 and 489/490 nm, indicating the presence of carotenoids (see Supplementary Fig. S3a in IJSEM Online). Similar absorbance spectra have been reported for other species of the genera *Sphingomonas* and *Novosphingobium* (Busse *et al.*, 1999, 2003). In order to characterize the pigment composition of strain FSW06-204d^T in comparison to other species of the genera *Novosphingobium*, *Sphingomonas* and *Sphingopyxis* in greater detail, several strains were grown in K7 medium at 25 °C. Pigments extracted with methanol/acetone (7/2, v/v) were separated by reverse-phase HPLC (HP 1100 Series; Hewlett Packard) on a C18 silica column (Multophyp ODS 5 μ m, 250 $\times$ 4.6 mm; CS Chromatographie Service) using a modification of method B as previously described by Airs *et al.* (2001). In brief, the mobile phase containing 5 % ammonium acetate, 80 % methanol and 15 % acetonitrile

was changed to 19 % methanol, 1 % acetonitrile and 80 % ethylacetate, thereby eluting apolar compounds. The gradient program was shortened from 85 to 40 min using a flow rate of 0.5 ml min⁻¹, yielding sufficient separation of carotenoids (see Supplementary Fig. S3b). The most abundant carotenoid of strain FSW06-204d^T was also most abundant in all of the investigated species of the genus *Novosphingobium* and *Sphingomonas parapaucimobilis* DSM 7463^T, but was not present in *Erythrobacter longus* ATCC 33941^T (see Supplementary Fig. S3b). Comparison of absorption spectra and retention times in HPLC analysis (see Supplementary Fig. S3c and Supplementary Table S1) suggests that this carotenoid represents nostoxanthin, the major carotenoid in *Sphingomonas paucimobilis* (Jenkins *et al.*, 1979) and *N. capsulatum* (Bally *et al.*, 1990). Another pigment of strain FSW06-204d^T (Peak 3, Supplementary Fig. S3b) was also common in all investigated species (see Supplementary Table S1) and was identified as bacteriorubixanthin by its presence in *E. longus* (Kim *et al.*, 2007). As *N. nitrogenifigens* DSM 19370^T was slightly yellow-pigmented, only the major pigments were present in HPLC chromatograms (see Supplementary Table S1). Strain FSW06-204d^T could be distinguished from other *Novosphingobium* species by the presence of an unidentified carotenoid (Peak 8, Supplementary Fig. S3b) in relatively high amounts (13.1 %, Supplementary Table S1).

Description of *Novosphingobium acidiphilum* sp. nov.

Novosphingobium acidiphilum (a.ci.di'phi.lum. N.L. neut. n. *acidum* an acid; Gr. adj. *philos* loving; N.L. neut. adj. *acidiphilum* acid-loving).

The species shares all of the characteristics of the genus as summarized by Takeuchi *et al.* (2001). Cells are rod-shaped, 1.4 $\pm$ 0.4 μ m long and 0.58 $\pm$ 0.06 μ m wide in the mid-exponential phase and motile in the early exponential phase. Growth is observed on YPG, K7 and R2A agars, but not on nutrient, malt, PYE, CASO, McConkey, soil extract, PCA, Czapek-Dox or LB agars. In liquid media, growth is observed at 25 °C in YPG, K7, R2A, M65, TS and NB media, but not in TSA or LB media. On YPG agar adjusted to pH 5.5, small circular yellow colonies are formed after incubation for 2–3 days at 25 °C and colonies turn orange after additional incubation for 3 to 5 days. Cells are Gram-negative, positive for cytochrome oxidase and catalase-negative. Obligately aerobic and able to grow under microaerophilic conditions. Endospores or capsules are not observed. Growth occurs between 4 and 32 °C, but not at 37 or 42 °C. Growth occurs between pH values of 4.5 and 7.0 in MES buffered K7 medium, but not at pH 3.5 or 7.5. At pH 5.5, growth is observed with up to 0.5 % NaCl (w/v), but not with 0.75 % NaCl (w/v). Best growth conditions in K7 medium are pH 5.5 to 6.0, 25 °C and without additional NaCl, with doubling times between 5.3 to 6.5 h during exponential growth. Hydrolyses aesculin, L-alanine-pNA, L-glutamate- γ -carboxy-pNA and L-proline-pNA. Gives a positive result in tests for the assimilation of

cellobiose, D-galactose, D-glucose, D-mannose, maltose, L-rhamnose, sucrose, L-malate, 2-oxoglutarate and L-proline and is slightly positive for the assimilation of L-arabinose, D-fructose and L-leucine. Negative result in tests for the assimilation of D-xylose, D-mannitol, citrate, DL-lactate, L-histidine and L-phenylalanine. Polar lipid profiles consist of phosphatidylmethylethanolamine and ethanolamine as major compounds, moderate amounts of a highly hydrophobic yellow pigment stainable with α -naphthol, diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylglycerol, sphingoglycolipid, an unknown highly hydrophilic aminophosphoglycolipid and two unknown polar lipids, and minor to trace amounts of one unknown glycolipid, two unknown phospholipids, two unknown polar lipids, another highly hydrophobic yellow pigment. No phosphatidylcholine is present and only a moderate content of sphingoglycolipids. The major fatty acid is C_{18:1} ω 7c and two 2-hydroxy fatty acids are present, C_{14:0} 2-OH and C_{15:0} 2-OH. The major quinone is ubiquinone Q-10 (90%). The predominant polyamine is spermidine (31.8 $\mu\text{mol g}^{-1}$ dry weight) and small amounts of putrescine (0.1 $\mu\text{mol g}^{-1}$ dry weight) and spermine (0.2 $\mu\text{mol g}^{-1}$ dry weight) are present. Acetone/methanol (7/2, v/v) extracted pigments are characterized by λ_{max} of 453 and 480 nm and cell-free extracts by λ_{max} at 432/433, 458 and 489/490 nm. The main carotenoid is nostoxanthin. In addition, seven minor carotenoids are present, including bacteriorubixanthin. The abundance of one unidentified carotenoid distinguishes the type strain from other species of the genus *Novosphingobium*. The 16S rRNA gene sequence of the type strain contains the specific nucleotide signatures defined for the genus *Novosphingobium* and exhibits a 21 bp signature gap also present in *N. nitrogenifigens* DSM 19370^T.

The type strain, FSW06-204d^T (=DSM 19966^T=CCM 7496^T=CCUG 55538^T) was isolated from subsurface water of the south-west basin of the acidic humic acid rich lake Grosse Fuchskuhle, north-eastern Germany.

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References

- Addison, S. L., Foote, S. M., Reid, N. M. & Lloyd-Jones, G. (2007). *Novosphingobium nitrogenifigens* sp. nov., a polyhydroxyalkanoate-accumulating diazotroph isolated from a New Zealand pulp and paper wastewater. *Int J Syst Evol Microbiol* 57, 2467–2471.
- Airs, R. L., Atkinson, J. E. & Keely, B. J. (2001). Development and application of a high resolution liquid chromatographic method for

the analysis of complex pigment distributions. *J Chromatogr A* 917, 167–177.

Allgaier, M. & Grossart, H.-P. (2006). Seasonal dynamics and phylogenetic diversity of free-living and particle-associated bacterial communities in four lakes in northeastern Germany. *Aquat Microb Ecol* 45, 115–128.

Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* 47, 39–52.

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* 43, 260–296.

Balkwill, D. L., Drake, G. R., Reeves, R. H., Frederickson, J. K., White, D. C., Ringelberg, D. B., Chandler, D. P., Romine, M. F., Kennedy, D. W. & Spadoni, C. M. (1997). Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *Int J Syst Bacteriol* 47, 191–201.

Bally, R., Givaudan, A., Bernillon, J., Heulin, T., Balandreau, J. & Bardin, R. (1990). Numerical taxonomic study of three N₂-fixing yellow-pigmented bacteria related to *Pseudomonas paucimobilis*. *Can J Microbiol* 36, 850–855.

Bartscht, K., Cypionka, H. & Overmann, J. (1999). Evaluation of cell activity and of methods for the cultivation of bacteria from a natural lake community. *FEMS Microbiol Ecol* 28, 249–259.

Brosius, J., Dull, T., Sleeter, D. & Noller, H. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 148, 107–127.

Burkert, U., Warnecke, F., Babenzien, D., Zwirnmann, E. & Pernthaler, J. (2003). Members of a readily enriched betaproteobacterial clade are common in surface waters of a humic lake. *Appl Environ Microbiol* 69, 6550–6559.

Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* 11, 1–8.

Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterization of *Sphingomonas*. *J Ind Microbiol Biotechnol* 23, 242–251.

Busse, H.-J., Denner, E. B. M., Buczolits, S., Salkinoja-Salonen, M., Bennisar, A. & Kämpfer, P. (2003). *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. *Int J Syst Evol Microbiol* 53, 1253–1260.

Fujii, K., Kikuchi, S., Satomi, M., Ushio-Sata, N. & Morita, N. (2003). *Novosphingobium tardagens* sp. nov., an oestradiol-degrading bacterium isolated from activated sludge of a sewage treatment plant in Tokyo. *Int J Syst Evol Microbiol* 53, 47–52.

Gich, F. & Overmann, J. (2006). *Sandarakinorhabdus limnophila* gen. nov., sp. nov., a novel bacteriochlorophyll a-containing, obligately aerobic bacterium isolated from freshwater lakes. *Int J Syst Evol Microbiol* 56, 847–854.

Glöckner, F. O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A. & Amann, R. (2000). Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of *Actinobacteria*. *Appl Environ Microbiol* 66, 5053–5065.

Jaspers, E., Nauhaus, K., Cypionka, H. & Overmann, J. (2001). Multitude and temporal variability of ecological niches as indicated by the diversity of cultivated bacterioplankton. *FEMS Microbiol Ecol* 36, 153–164.

- Jenkins, C. L., Andrewes, A. G., McQuade, T. J. & Starr, M. D. (1979). The pigment of *Pseudomonas paucimobilis* is a carotenoid (nostoxanthin), rather than a brominated aryl-polyene (xanthomonadin). *Curr Microbiol* **3**, 1–4.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Witzemberger, R., Denner, E. B. M., Busse, H.-J. & Neef, A. (2002). *Novosphingobium hassiacum* sp. nov., a new species isolated from an aerated sewage pond. *Syst Appl Microbiol* **25**, 37–45.
- Kim, M. K., Schubert, K., Im, W.-T., Kim, K.-H., Lee, S.-T. & Overmann, J. (2007). *Sphingomonas kaistensis* sp. nov., a novel alphaproteobacterium containing *pufLM* genes. *Int J Syst Evol Microbiol* **57**, 1527–1534.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Liu, Z.-P., Wang, B.-J., Liu, Y.-H. & Liu, S.-J. (2005). *Novosphingobium taihuense* sp. nov., a novel aromatic-compound-degrading bacterium isolated from Taihu Lake, China. *Int J Syst Evol Microbiol* **55**, 1229–1232.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Neef, A., Witzemberger, R. & Kämpfer, P. (1999). Detection of sphingomonads and *in situ* identification in activated sludge using 16S rRNA-targeted oligonucleotide probes. *J Ind Microbiol Biotechnol* **23**, 261–267.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Rainey, F. A., Ward-Rainey, N., Gliesche, C. G. & Stackebrandt, E. (1998). Phylogenetic analysis and intrageneric structure of the genus *Hyphomicrobium* and the related genus *Filomicrobium*. *Int J Syst Bacteriol* **48**, 635–639.
- Sohn, J. H., Kwon, K. K., Kang, J.-H., Jung, H.-B. & Kim, S.-J. (2004). *Novosphingobium pentaromativorans* sp. nov., a high-molecular-mass polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment. *Int J Syst Evol Microbiol* **54**, 1483–1487.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.
- Suzuki, S. & Hiraishi, A. (2007). *Novosphingobium naphthalenivorans* sp. nov., a naphthalene-degrading bacterium isolated from polychlorinated-dioxin-contaminated environments. *J Gen Appl Microbiol* **53**, 221–228.
- Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.
- Tirola, M. A., Männistö, M. K., Puhakka, J. A. & Kulomaa, M. S. (2002). Isolation and characterization of *Novosphingobium* sp. strain MT1, a dominant polychlorophenol-degrading strain in a ground-water bioremediation system. *Appl Environ Microbiol* **68**, 173–180.
- Tirola, M. A., Busse, H.-J., Kämpfer, P. & Männistö, M. K. (2005). *Novosphingobium lentum* sp. nov., a psychrotolerant bacterium from a polychlorophenol bioremediation process. *Int J Syst Evol Microbiol* **55**, 583–588.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tschech, A. & Pfennig, N. (1984). Growth yield increase linked to caffeate reduction in *Acetobacterium woodii*. *Arch Microbiol* **137**, 163–167.
- Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99–119.