Novosphingobium taihuense sp. nov., a novel aromatic-compound-degrading bacterium isolated from Taihu Lake, China

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A novel aromatic-compound-degrading bacterium, strain T3-B9^T, was isolated from sediment of Taihu Lake, Jiangsu Province, south-east China. This bacterial isolate assimilated several aromatic compounds such as phenol, aniline, nitrobenzene, 4-chloronitrobenzene and phenanthrene. The taxonomy of strain T3-B9^T was studied by polyphasic methods. The organism showed a range of phenotypic and chemotaxonomic properties consistent with those of the genus *Novosphingobium*. The 16S rRNA gene sequence similarity of strain T3-B9^T to members of the genus *Novosphingobium* ranged from 91.6 to 97.5%, and this isolate clustered phylogenetically with members of genus *Novosphingobium*. The DNA–DNA relatedness values of strain T3-B9^T to the most phylogenetically related species, *Novosphingobium subterraneum* DSM 12447^T, *Novosphingobium aromaticivorans* ATCC 700278^T and *Novosphingobium stygium* ATCC 700280^T, were 31, 33 and 14%, respectively. The combined genotypic and phenotypic data show that strain T3-B9^T represents a novel species of the genus *Novosphingobium*, for which the name *Novosphingobium taihuense* sp. nov. is proposed. The type strain is T3-B9^T (=AS 1.3432^T=JCM 12465^T).

The genus *Novosphingobium* was created by Takeuchi *et al.* (2001) to accommodate species of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990) that clustered phylogenetically with *Novosphingobium capsulatum* [cluster III of Takeuchi *et al.* (2001)], have 2-OH 14:0 as the major 2-hydroxy fatty acid and spermidine as the major polyamine and are able to reduce nitrate (Takeuchi *et al.*, 2001). Although Yabuuchi *et al.* (2002) argued that the creation of the genus *Novosphingobium* required more phenotypic support, many sphingomonad taxonomists (Kämpfer *et al.*, 2002; Fujii *et al.*, 2003; Sohn *et al.*, 2004; Tirola *et al.*, 2005) adhere to the proposal of Takeuchi *et al.* (2001) because of phylogenetic and also chemotaxonomic studies that clearly separate *Novosphingobium* from the genus *Sphingomonas sensu stricto*. Currently, the genus *Novosphingobium* includes the following species with validly published names: *Novosphingobium aromaticivorans* (Balkwill *et al.*, 1997), *Novosphingobium capsulatum* (Leifson, 1962; Yabuuchi *et al.*, 1990), *Novosphingobium hassiacum* (Kämpfer *et al.*, 2002), *Novosphingobium pentaromaticivorans* (Sohn *et al.*, 2004),

Novosphingobium rosa (Takeuchi *et al.*, 1995), *Novosphingobium stygium* (Balkwill *et al.*, 1997), *Novosphingobium subarcticum* (Nohynek *et al.*, 1996), *Novosphingobium subterraneum* (Balkwill *et al.*, 1997), *Novosphingobium tardaogens* (Fujii *et al.*, 2003) and *Novosphingobium lentum* (Tirola *et al.*, 2005). The organisms of this genus are inhabitants of soil, sediments and other subsurfaces and are able to degrade various xenobiotic aromatic compounds (Stevens *et al.*, 1993; Fredrickson *et al.*, 1995; Karlson *et al.*, 1995; Kim *et al.*, 1996; Wildes *et al.*, 1996; Balkwill *et al.*, 1997). Thus, they are potentially important in the biogeochemical cycles of carbon, nitrogen or chlorine, elements of xenobiotic compounds in their surrounding environments. They have also potential biotechnological applications, for example, the bioremediation of aromatic-contaminated environments.

During a systematic investigation of the microbial ecology and elemental biogeochemistry of Taihu Lake, located in south-east China, a yellow-pigmented bacterial strain, designated strain T3-B9^T, was isolated from a lake sediment sample (GPS location of sampling site is 120° 02' 16.8" E 31° 27' 10.7" N). Sediment samples were obtained at 10 cm beneath the sediment surface. Strain T3-B9^T was isolated by plating 0.1 ml of 10-fold dilutions of the sample onto diluted LB agar (0.1% peptone, 0.05% yeast extract, 0.1% NaCl, 1.5% agar). Routine cultivation was conducted aerobically with LB agar or LB broth at 30 °C.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T3-B9^T is AY500142.

The polar lipid profile of strain T3-B9^T and a phylogenetic tree for *Novosphingobium* species constructed using the maximum-parsimony method are available as supplementary material in IJSEM Online.

Gram staining was conducted according to the method described by Gerhardt *et al.* (1994). Oxidase activity, indole production and hydrolysis of starch etc. were tested according to the methods described by Dong & Cai (2001). Spore formation was determined by malachite green staining of cells grown on LB agar. Flagellation was examined by using a transmission electron microscope (H-600; Hitachi) at 100 kV after negative staining with 1 % (w/v) phosphotungstic acid. Morphological observation was carried out using a scanning electron microscope (FEI Quanta 2000). For assaying assimilation of aromatic compounds, each compound was added at a concentration of 30 mg l⁻¹ to mineral medium broth (Liu *et al.*, 2002). Growth was examined after incubation at 30 °C for 1 week. Denitrification was determined by the method of Stanier *et al.* (1966). Catalase activity was judged by formation of bubbles after a 3 % H₂O₂ solution was dropped onto a fresh colony. Aerobic and anaerobic production of acids (OF reaction) from carbohydrates was determined in OF basal medium (Hugh & Leifson, 1953). Carbohydrate solutions, sterilized by filtration, were added at a final concentration of 1 % (w/v) and acid production from carbohydrates was recorded after 7 and 14 days of incubation. Cellular fatty acids were extracted from cells grown in LB broth at 30 °C and subsequently purified and analysed as described previously (Hu *et al.*, 2004 and papers cited therein). For the analysis of polar lipids and quinones, strain T3-B9^T was grown at 22 °C in R2A broth; cells were harvested at the late-exponential phase, centrifuged and lyophilized. Polar lipids were examined by two-dimensional TLC and characterized with spreading reagents specific for α -glycols (periodate-Schiff), sugars (α -naphthol-H₂SO₄, anisaldehyde-H₂SO₄) and phosphate (Zindzadze) (Ventosa *et al.*, 1993). Quinones were determined according to Collins (1985) and Wu *et al.* (1989). DNA base compositions were determined by thermal denaturation (Marmur & Doty, 1962) and DNA from *Escherichia coli* DH-5 α was used as standard for the calibration of the *T*_m value. The 16S rRNA gene was amplified and sequenced as described previously (Zhang *et al.*, 2003). 16S rRNA gene sequence alignments were performed using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were constructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods. DNA-DNA hybridization was performed by the thermal denaturation and renaturation method of De Ley *et al.* (1970) and modified by Huß *et al.* (1983).

Cells of strain T3-B9^T were Gram-negative, aerobic, non-spore-forming, non-motile rods, about 0.45 × 1.8 ~ 4.0 µm. The strain produced small (about 2 mm), yellow colonies on 10-fold-diluted LB agar, LB agar or R2A agar after 3 days cultivation. The optimal temperature for growth of strain T3-B9^T was 25 °C; growth was observed at 10–37 °C, but not at 4 or 40 °C. The optimal pH for growth was 6.0; growth occurred at pH 4.5–8.5, but not at pH 4.0 or 9.0. It assimilated the following aromatic compounds for growth: phenol, 3-aminophenol, aniline,

nitrobenzene, 4-chloronitrobenzene, 3-hydroxybenzoate and phenanthrene, but not 3- or 4-chloroaniline, chlorobenzene, 3,5-dihydroxytoluene, anthracene or pyrene.

Strain T3-B9^T reduced nitrate, but did not produce N₂, consistent with the properties of the genus *Novosphingobium* (Takeuchi *et al.*, 2001). H₂S was not produced. Catalase activity was positive. Additional features and some properties that differentiate strain T3-B9^T from other members of the genus *Novosphingobium* are given in Table 1. Strain T3-B9^T contained 18:1 as the dominant fatty acid and 2-OH 14:0 as the major hydroxylated fatty acid. The cellular fatty acid profile of strain T3-B9^T is outlined in the species description. Strain T3-B9^T contained

Table 1. Differentiation of strain T3-B9^T from other *Novosphingobium* species

Strains: 1, T3-B9^T; 2, *N. hassiacum* DSM 14552^T (data from Kämpfer *et al.*, 2002); 3, *N. tardaugens* JCM 11434^T (Fujii *et al.*, 2003); 4, *N. subarcticum* JCM 10398^T; 5, *N. subterraneum* DSM 12447^T; 6, *N. stygium* ATCC 700280^T (data for columns 4–6 from Balkwill *et al.*, 1997); 7, *N. capsulatum* ATCC 14666^T (Kämpfer *et al.*, 1997); 8, *N. rosa* ATCC 51837^T (Kämpfer *et al.*, 1997); 9, *N. aromaticivorans* ATCC 700278^T (Balkwill *et al.*, 1997); 10, *N. pentaromaticivorans* KCTC 10454^T (Sohn *et al.*, 2004); 11, *N. lentum* DSM 13663^T (Tirola *et al.*, 2005). +, Positive; –, negative; (+), weakly positive; ND, not detected or not described. All strains were positive for catalase and nitrate reduction. All strains were negative for arginine dihydrolase and urease activity, production of indole, glucose fermentation, denitrification and assimilation of adonitol, β -alanine, citrate, D-sorbitol, L-phenylalanine, phenylacetate and D-ribose.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Assimilation of:											
N-Acetyl-D-glucosamine	–	–	–	+	–	–	+	(+)	+	–	–
L-Arabinose	+	–	–	+	–	–	(+)	+	–	–	–
D-Cellobiose	+	+	–	–	+	–	+	+	+	–	–
D-Fructose	+	–	–	–	–	–	–	+	–	+	–
D-Galactose	–	–	–	+	–	–	+	–	–	–	–
D-Glucose	+	(+)	–	+	+	–	+	+	+	–	–
D-Mannose	+	–	–	–	+	–	(+)	+	+	–	–
D-Maltose	+	–	–	+	+	–	+	+	+	–	–
D-Melibiose	–	–	–	–	+	–	–	+	–	–	–
L-Rhamnose	+	–	–	+	+	–	+	+	+	+	–
Sucrose	+	–	–	–	+	–	+	+	+	+	–
D-Trehalose	–	–	–	–	–	–	+	–	–	+	–
D-Xylose	+	–	–	+	+	–	+	+	+	–	–
D-Mannitol	–	–	–	+	–	+	–	–	–	ND	–
L-Alanine	+	–	–	+	–	–	–	–	–	–	–
L-Proline	+	+	–	+	+	–	–	–	+	+	–
Hydrolysis of:											
Gelatin	–	+	–	+	–	–	–	–	–	–	–
Aesculin	+	–	ND	+	+	+	+	(+)	+	+	–
Oxidase activity	–	+	–	–	–	–	–	–	–	–	–

sphingoglycolipid, phosphatidylethanolamine and diphosphatidylglycerol as major identified polar lipids (see Supplementary Fig. A in IJSEM Online). The unidentified phospholipid PLx1 was most likely identical to the corresponding unidentified phospholipids of *N. lentum* (PLx1; Tirola *et al.*, 2005) and of *N. hassiacum* (PL; Kämpfer *et al.*, 2002). The unidentified polar lipids Lx1 and PLx2 of strain T3-B9^T were most likely identical to the corresponding polar lipids Lx1 and PLx2 of *N. lentum* (Tirola *et al.*, 2005). Strain T3-B9^T showed more unidentified polar lipids, Lx2 and Lx3, when compared to *N. lentum* (Tirola *et al.*, 2005), and phosphatidylmonomethylethanolamine (PME) was not detected. The combined presence of unknown polar lipids and the absence of PME clearly distinguishes T3-B9^T from other species of *Novosphingobium*, including *N. aromaticivorans*, *N. capsulatum*, *N. stygium*, *N. subterraneum*, *N. subarcticum*, *N. rosa* (Busse *et al.*, 1999), *N. hassiacum* (Kämpfer *et al.*, 2002) and *N. lentum* (Tirola *et al.*, 2005). The predominant isoprenoid quinone of strain T3-B9^T was Q-10. The G + C content of strain T3-B9^T was 63.3 mol% (T_m), which is in accordance with the range described for the genus *Novosphingobium* (62–67 mol%; Takeuchi *et al.*, 2001). All these data indicate that strain T3-B9^T is a member of the genus *Novosphingobium*.

The sequence of the 16S rRNA gene of strain T3-B9^T was aligned with others of members of the genus *Novosphingobium* and results showed the signature nucleotides of *Novosphingobium*: 52C, 134G, 359G, 593U, 987G, 990U, 1215A and 1218C (Takeuchi *et al.*, 2001). Strain T3-B9^T showed levels of 16S rDNA gene sequence (1362 base pairs) similarity ranging from 91.6 to 97.5% to members of the genus *Novosphingobium*, with the highest to *N. subterraneum* DSM 12447^T (97.5%) and *N. aromaticivorans* ATCC 700278^T (97.2%). A neighbour-joining tree (Fig. 1) showed that strain T3-B9^T formed coherent clades with other members of the genus *Novosphingobium* and it clustered closely with *N. stygium* ATCC 700280^T (75% bootstrap support). A tree derived by the maximum-parsimony method is available as Supplementary Fig. B in IJSEM Online. The DNA–DNA relatedness of strain T3-B9^T to *N. subterraneum* DSM 12447^T, *N. aromaticivorans* ATCC 700278^T and *N. stygium* ATCC 700280^T was 31, 33 and 14%, respectively.

Combining the above phenotypic, chemotaxonomic and genotypic results, we conclude that strain T3-B9^T represents a novel species of the genus *Novosphingobium*, for which the name *Novosphingobium taihuense* is proposed.

Description of *Novosphingobium taihuense* sp. nov.

Novosphingobium taihuense (tai.hu.en'se. N.L. neut. adj. *taihuense* pertaining to Taihu Lake, the source of the sediment from which the type strain was isolated).

Cells are Gram-negative, aerobic, non-spore-forming, non-motile rods. Produces small (about 2 µm), yellow colonies

Fig. 1. Phylogenetic tree constructed with the neighbour-joining method based on 16S rRNA gene sequence alignments of strain T3-B9^T and related bacteria. Numbers at nodes indicate percentage levels of bootstrap support based on 1000 resampled datasets; only values greater than 50% are shown. *Sphingomonas suberifaciens* IFO 15211^T was used as the outgroup. Bar, evolutionary distance (K_{nuc}) of 0.1. A maximum-parsimony tree is available as supplementary material in IJSEM Online.

on 10-fold dilution of LB agar, LB agar or R2A agar, after incubation at 30 °C for 3 days. The colonies are smooth, shiny, flat, non-transparent, elevated and rubbery. Growth occurs at 10–37 °C, pH 4.5–8.5 and 0–2.0% NaCl; optimal growth temperature, pH and salinity are 25 °C, pH 6.0 and 0.5% NaCl. Positive for catalase activity and nitrate reduction. Activity of oxidase, urease and arginine dihydrolase, production of indole, growth on citrate, hydrolysis of starch and gelatin and glucose fermentation are all negative. Other characteristics are listed in Table 1. The dominant fatty acid is 18:1 (36.5%) and the major hydroxylated fatty acid is 2-OH 14:0 (12.8%). The fatty acid profile also contains 15:0 (2.4%), 16:0 (9.9%), 16:1 (11.9%), 17:0 (1.9%), 17:1 (17.3%), 18:0 (0.7%), 13:0 2-OH (0.9%), 2-OH 15:0 (4.6%) and 2-OH 16:0 (0.6%). The predominant isoprenoid quinone is Q-10. Cells contain phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and an unknown polar lipid as major polar lipids; it also contains phosphatidylcholine, unknown phosphoglycolipids and other unknown polar lipids or phospholipids. DNA G + C content is 63.3 mol% (T_m).

The type strain is T3-B9^T (= AS 1.3432^T = JCM 12465^T), isolated from sediment of Taihu Lake, Jiangsu Province, China.

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