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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{T} to the most phylogenetically related species, Novosphingobium subterraneum DSM 12447\textsuperscript{T}, Novosphingobium aromaticivorans ATCC 700278\textsuperscript{T} and Novosphingobium stygium ATCC 700280\textsuperscript{T}, were 31, 33 and 14\%, respectively. The combined genotypic and phenotypic data show that strain T3-B9\textsuperscript{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\textsuperscript{T} (=AS 1.3432\textsuperscript{T} = JCM 12465\textsuperscript{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; Tiirola 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 pentaromativorans (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 tardagens (Fujii et al., 2003) and Novosphingobium lentum (Tiirola 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\textsuperscript{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\textsuperscript{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.
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-B9ᵀ 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 x-glycols (periodate-Schiff), sugars (x-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-5α was used as standard for the calibration of the Tm 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-B9ᵀ were Gram-negative, aerobic, non-sporing, 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-B9ᵀ was 25 °C; growth was observed at 10–37 °C, but not at 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-B9ᵀ 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-B9ᵀ from other members of the genus Novosphingobium are given in Table 1.

The cellular fatty acid profile of strain T3-B9ᵀ is outlined in the species description. Strain T3-B9ᵀ contained

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Table 1. Differentiation of strain T3-B9ᵀ from other Novosphingobium species

| Strains: 1, T3-B9ᵀ; 2, N. hussiacum DSM 14552ᵀ (data from Kämpfer et al., 2002) 3, N. tardaugens JCM 11434ᵀ (Fujii et al., 2003); 4, N. subarcticum DSM 10398ᵀ; 5, N. subterraneum DSM 12447ᵀ; 6, N. stygiomyces ATCC 700280ᵀ (data for columns 4–6 from Balkwill et al., 1997); 7, N. capsulatum ATCC 14666ᵀ (Kämpfer et al., 1997); 8, N. rosa ATCC 51837ᵀ (Kämpfer et al., 1997); 9, N. aromaticivorans ATCC 700278ᵀ (Balkwill et al., 1997); 10, N. pentaromativorans KCTC 10454ᵀ (Sohn et al., 2004); 11, N. lentum DSM 13663ᵀ (Tiitola 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.

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sphingoglycolipid, phosphatidylethanolamine and diphasphatidylglycerol 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; Tiirila 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* (Tiirila et al., 2005). Strain T3-B9\(^T\) showed more unidentified polar lipids, Lx2 and Lx3, when compared to *N. lentum* (Tiirila 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* (Tiirila 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*, 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* (Tiirila 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*.

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* (tail.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 mm), yellow colonies 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 % 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 phosphatidic acid, monophosphatidylglycerol 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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References


