**Sphingobium jiangsuense** sp. nov., a 3-phenoxybenzoic acid-degrading bacterium isolated from a wastewater treatment system

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A non-sporulating, non-motile, catalase- and oxidase-positive, Gram-negative, rod-shaped bacterial strain, designated BA-3, was isolated from activated sludge of a wastewater treatment facility. The strain was able to degrade about 95 % of 100 mg 3-phenoxybenzoic acid l⁻¹ within 2 days of incubation. Growth occurred in the presence of 0–2 % (w/v) NaCl (optimum, 0.5 % (w/v) NaCl), at pH 5.5–9.0 (optimum, pH 7.0) and at 10–37 °C (optimum, 28 °C). Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain BA-3 was a member of the genus *Sphingobium*; it showed highest gene sequence similarity to *Sphingobium qiguonii* X23 (98.2 %), and similarities of <97.0 % with strains of other *Sphingobium* species. The polar lipid pattern, the presence of spermidine and ubiquinone Q-10, the predominance of summed feature 8 (C₁₈:1ω6c and/or C₁₈:1ω7c) in the cellular fatty acid profile and the DNA G+C content also supported affiliation of the isolate to the genus *Sphingobium*. Strain BA-3 showed low DNA–DNA relatedness values (21.3 ± 0.8 %) with *Sphingobium qiguonii* X23. Based on phenotypic, genotypic and phylogenetic data, strain BA-3 represents a novel species of the genus *Sphingobium*, for which the name *Sphingobium jiangsuense* sp. nov. is proposed; the type strain is BA-3 (=CCTCC AB 2010217T=KCTC 23196=KACC 16433).

The genus *Sphingobium* was proposed by Takeuchi et al. (2001) after the subdivision of the genus *Sphingomonas* (Yabuuchi et al., 1990; Takeuchi et al., 1993), due to the latter containing genetically relatively diverse species based on 16S rRNA gene sequence analysis. The genus *Sphingobium* is represented by environmental isolates that degrade a wide variety of xenobiotic pollutants (Ushiba et al., 2003; Pal et al., 2005; Prakash & Lal, 2006; Wittich et al., 2007; Dadhwal et al., 2009; Kumari et al., 2009; Singh & Lal, 2009; Wang et al., 2009, 2011; Bala et al., 2010; Guo et al., 2010; Liang & Lloyd-Jones, 2010; Yan et al., 2010). At the time of writing, the genus *Sphingobium* comprised 25 species. In this paper, a 3-phenoxybenzoic acid (3-POB)-degrading bacterial strain, designated BA-3, was isolated from activated sludge of a wastewater treatment plant in a synthetic pyrethroid manufacturing facility in Yangzhou Jiangsu province, China (32° 24’ N 119° 26’ E), by an enrichment procedure. The mineral salts medium (MSM) for primary enrichment culture contained (l⁻¹) 4.8 g K₂HPO₄, 1.2 g KH₂PO₄, 1.0 g NH₄NO₃, 0.2 g MgSO₄·7H₂O, 0.4 g Ca(NO₃)₂·4H₂O, 0.5 g NaCl and 0.001 g Fe₂(SO₄)₃, with 100 mg 3-POB as the sole carbon source; for MSM plates, 1.5 % agar was added. Sludge (1.0 g sample) was inoculated into 50 ml MSM and inoculated at 30 °C for 6 days. After five successive steps of subcultivation, the enrichment culture was spread on MSM plates and incubated at 30 °C for 4–5 days. Strain BA-3 was obtained after several streakings and transfers on MSM plates. The taxonomic status of this strain was determined using a polyphasic taxonomic approach. The data obtained suggest that the isolate represents a novel species of the genus *Sphingobium*.

*Sphingobium qiguonii* X23 (Yan et al., 2010) and *Sphingobium yanoikuyae* LMG 11252T (type species of the genus), used as controls in phenotypic and chemotaxonomic studies, were kindly provided by Q. Hong, Nanjing Agricultural University, and obtained from the Belgian Co-ordinated Collections of Micro-Organisms (BCCM/LMG), respectively. Unless indicated otherwise, morphological, physiological, biochemical and molecular studies were...
performed with cultures grown aerobically on 10-fold-diluted Luria–Bertani (LB) agar or in 10-fold-diluted LB broth at 28°C for 3 days. The 10-fold-diluted LB medium was composed of (L−1) 1.0 g tryptone, 0.5 g yeast extract and 1.0 g NaCl.

Cell morphology and dimensions were determined by transmission electron microscopy (H-7650; Hitachi). In preparation for electron microscopy, bacterial cells were suspended in 0.85% NaCl. Subsequently, the cells were dried on a nickel-coated mesh, negatively stained with phosphotungstic acid and examined. The motility of cells was tested by the hanging drop method (Skerman, 1967). The Gram reaction was determined by the KOH test (Buck, 1982) and further confirmed by the conventional Gram-staining method (Gregersen, 1978). The pH range (pH 5.0–10.0, at intervals of 0.5) for growth was studied using citrate/phosphate buffer or Tris/HCl buffer (Breznak & Costilow, 1994). Growth at various temperatures (5, 10, 15, 25, 28, 30, 37, 40, 41 and 45°C) and NaCl concentrations [final concentrations of 0–5.0% (w/v) at increments of 0.5%] was determined in 10-fold-diluted LB broth. Catalase activity was determined as described previously (Ohta & Hattori, 1983). Nitrate reduction, H2S production, indole production, urease and gelatinase tests, and assimilation and oxidation of various carbon compounds were performed using the API 20NE kit (bioMérieux), the API 20E kit (bioMérieux) and the Biolog GN2 system following the manufacturer’s recommendations. Anaerobic growth was tested in 10-fold-diluted LB broth containing thioglycolate (1 g L−1) in a serum bottle with the headspace substituted with nitrogen gas. The ability to degrade 3-POB was determined as described by Guo et al. (2009); 3-POB was added at a final concentration of 100 mg L−1.

The fatty acid profiles of strain BA-3T, S. qiguonii X23T and S. yanoikuyae LMG 11252T were determined using the Sherlock Microbial Identification System according to manufacturer’s instructions (MIDI Corporation) (Sasser, 1990). All strains were streaked on tryptic soy broth agar plates and cultured for 36/48 h (S. yanoikuyae LMG 11252T was cultured for 36 h, strains BA-3T and S. qiguonii X23T were cultured for 48 h). Cells were harvested from the third quadrant of the quadrant-streaked plate. Fatty acid methyl esters were obtained from cells by saponification, methylation and extraction, and separated by GC (Agilent 6890N). Peaks were automatically integrated and fatty acid names and percentages were determined using the MIDI Sherlock MIS system (TSBA6 library, version 6.0B). Polar lipid analyses were carried out by the Identification Service of the DSMZ and B. J. Tindall, DSMZ, Braunschweig, Germany (Minnikin et al., 1984; Collins & Jones, 1980). Isoprenoid quinones were extracted by using the method of Collins et al. (1977) and analysed by HPLC as described by Kroppenstedt (1982). Bacterial polyamines were analysed by HPLC according to Busse & Auling (1988) and Busse et al. (1997).

Bacterial DNA was purified using lysis solution containing 100 μg proteinase K ml−1 and 1% SDS, phenol/chloroform extractions and 2-propanol precipitation according to standard procedures (Sambrook & Russell, 2001). The nearly complete 16S rRNA gene sequence was obtained by PCR amplification using a set of universal primers, 5′-AGAGTTTGATCCTGTCAG-3′ (Escherichia coli bases 8–27) and 5′-TACCTTGTTACGACTT-3′ (E. coli bases 1507–1492), originally presented by Lane (1991). The PCR product was purified using a PCR purification kit (Axygen), ligated into vector pMD18-T (TaKaRa Biotechnology) and then transformed into E. coli DH5α. An automatic sequencer (model 3730; Applied Biosystems) was used to determine the 16S rRNA gene sequence. Pairwise sequence similarity was calculated by using a global alignment algorithm, implemented at the EzTaxon server (http://www.eztaxon.org; Chun et al., 2007). Phylogenetic analysis was performed by using the software package MEGA version 5.0 (Tamura et al., 2011) after multiple alignment of the sequence data with CLUSTAL_X (Thompson et al., 1997). Distances were calculated by using distance options according to Kimura’s two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (based on 1200 resamplings) (Felsenstein, 1985). The genomic DNA G+C content was determined by thermal denaturation (Mandel & Marmur, 1968) using E. coli K-12 as a standard.

An almost complete 16S rRNA gene sequence (1445 nt) of strain BA-3T was obtained. In the neighbour-joining and maximum-likelihood phylogenetic trees (Fig. 1; Supplementary Fig. S1, available in IJSEM Online), strain BA-3T grouped among Sphingobium species and formed a subclade with S. qiguonii X23T. Strain BA-3T showed highest 16S rRNA gene sequence similarity to S. qiguonii X23T (98.2%), and similarities of <97.0% with strains of other species of the genus Sphingobium. Strain BA-3T showed 95.9% similarity to S. yanoikuyae LMG 11252T, the type strain of the type species of the genus Sphingobium.

DNA–DNA hybridization is necessary to clarify the taxonomic relationship of strains when these share more than 97% 16S rRNA gene sequence similarity (Tindall et al., 2010). To further clarify the taxonomic relationship of strain BA-3T with S. qiguonii X23T, DNA–DNA hybridizations were performed according to the method of Ezaki et al. (1989). Hybridizations were repeated three times and means of the resulting values were determined; reciprocal experiments were also performed between strain BA-3T and S. qiguonii X23T. The results indicated that strain BA-3T showed low DNA–DNA relatedness to S. qiguonii X23T (21.3±0.8%: reciprocal, 28.9±1.3%). Supplementary Table S1, available in IJSEM Online); relatedness values were well below the threshold of 70% recommended for the delineation of bacterial species (Wayne et al., 1987).

Chemotaxonomically, strain BA-3T possessed chemical markers that supported its assignment to the genus

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Sphingobium. Cellular fatty acid analyses revealed that the major non-hydroxylated fatty acids (>5%) were summed feature 8 (C18:1ω6c and/or C18:1ω7c; 67.0%), C16:0 (10.3%) and summed feature 3 (C16:1ω6c and/or C16:1ω7c; 5.9%), and the major hydroxylated fatty acid was C14:0 2-OH (7.9%) (Table 1). The fatty acid profile of strain BA-3T differed from that of the other strains studied; compared to S. qiguonii X23T and S. yanoikuyae LMG 11252T, strain BA-3T possessed comparatively high levels of summed feature 8 (C18:1ω6c and/or C18:1ω7c) and low levels of summed feature 3 (C16:1ω6c and/or C16:1ω7c). C16:0 2-OH was present in S. yanoikuyae LMG 11252T, but was not found in strain BA-3T and S. qiguonii X23T. The major respiratory quinone was ubiquinone Q-10 (96%). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, two sphingoglycolipids, glycolipid and phosphatidylcholine (Supplementary Fig. S2, available in IJSEM Online). These polar lipids are commonly reported for members of the genus Sphingobium (Busse et al., 1999). Strain BA-3T contained 4.73 μmol spermidine (g wet cells)\(^{-1}\) as the predominant polyamine.

Strain BA-3T was examined for a range of phenotypic characteristics. The isolate was strictly aerobic and cells were Gram-negative, non-motile, non-sporulating and rod-shaped (0.6–0.8 6 1.0–1.2 μm). Colonies on 10-fold-diluted LB agar were cream-white, convex and circular with entire margins. The strain grew in the presence of 0–2 % (w/v) NaCl (optimum 0.5 %), at 10–37 °C (optimum 28 °C) and at pH 5.5–9.0 (optimum pH 7.0). Strain BA-3T was able to degrade about 95 % of 100 mg 3-POB l\(^{-1}\) within 2 days incubation (Supplementary Fig. S3, available in IJSEM Online). The DNA G+C content of strain BA-3T was 63.8 mol%, which fell within the range observed for other members of the genus Sphingobium (62–67 mol%, Takeuchi et al., 2001). Detailed morphological, physiological and biochemical characteristics of strain BA-3T are summarized in the species description below and in Table 2.

The predominance of summed feature 8 (C18:1ω6c and/or C18:1ω7c) in the cellular fatty acids, the detection of ubiquinone Q-10, the presence of sphingoglycolipid and the DNA G+C content (63.8 mol%) of strain BA-3T are
Table 1. Cellular fatty acid profiles of strain BA-3\textsuperscript{T} and strains of related species of the genus Sphingobium

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
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<tbody>
<tr>
<td>C\textsubscript{14}:0</td>
<td>1.1</td>
<td>0.0</td>
<td>1.1</td>
</tr>
<tr>
<td>C\textsubscript{16}:0</td>
<td>11.5</td>
<td>11.5</td>
<td>11.5</td>
</tr>
<tr>
<td>C\textsubscript{18}:0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C\textsubscript{16}:0\textsubscript{10}:5c</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>C\textsubscript{17}:0\textsubscript{10}:6c</td>
<td>2.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>C\textsubscript{18}:0\textsubscript{10}:5c</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>11-Methyl-C\textsubscript{18}:1\textsubscript{10}:7c</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>C\textsubscript{14}:0 2-OH</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
<tr>
<td>C\textsubscript{16}:0 2-OH</td>
<td>10.3</td>
<td>10.3</td>
<td>10.3</td>
</tr>
</tbody>
</table>

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 contains C\textsubscript{16}:0\textsubscript{10}:6c and/or C\textsubscript{16}:0\textsubscript{10}:7c; summed feature 8 contains C\textsubscript{18}:1\textsubscript{10}:6c and/or C\textsubscript{18}:1\textsubscript{10}:7c.

Table 2. Differential phenotypic characteristics of strain BA-3\textsuperscript{T} and related species of the genus Sphingobium

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Colony colour*</td>
<td>CW</td>
<td>GW</td>
<td>(Y)</td>
</tr>
<tr>
<td>Motility</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(\beta)-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*Determined after 5 days incubation on 10-fold-diluted LB agar. CW, Cream–white; GW, greyish white; (Y), yellowish.
phosphatidylethanolamine, two sphingoglycolipids, glycolipid and phosphatidylcholine. The major cellular polyamine is spermidine.

The type strain is BA-3T (=CCTCC AB 2010217T=KCTC 23196T=KACC 16433T), isolated from a wastewater treatment system in a pesticide manufacturing company in Jiangsu province, PR China. The DNA G+C content of the type strain is 63.8 mol%.

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References


