Sphingobium aromaticiconvertens sp. nov., a xenobiotic-compound-degrading bacterium from polluted river sediment

Rolf-Michael Wittich,1,2 Hans-Jürgen Busse,3 Peter Kämpfer,4 Marja Tirola,5 Monika Wieser,3 Alexandre J. Macedo1 and Wolf-Rainer Abraham1

Correspondence
Wolf-Rainer Abraham
wab@gbf.de

1Helmholtz Zentrum für Infektionsforschung (formerly GBF), Division Microbiology, D-38124 Braunschweig, Germany
2Estación Experimental del Zaidín – EEZ-CSIC, Depto de Bioquímica, Biología Celular y Molecular, Línea de Degradoación de Tóxicos Orgánicos, Calle Profesor Albareda 1, E-18008 Granada, Spain
3Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, A-1210 Wien, Austria
4Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität, D-35390 Giessen, Germany
5Department of Biological and Environmental Science, University of Jyväskylä, FIN-40100 Jyväskylä, Finland

A bacterial strain capable of degrading some monochlorinated dibenzofurans, designated RW16T, was isolated from aerobic River Elbe sediments. The strain was characterized based on 16S rRNA gene sequence analysis, DNA G+C content, physiological characteristics, polyamines, ubiquinone and polar lipid pattern and fatty acid composition. This analysis revealed that strain RW16T represents a novel species of the genus Sphingobium. The DNA G+C content of strain RW16T, 60.7 mol%, is the lowest yet reported for the genus. 16S rRNA gene sequence analysis placed strain RW16T as an outlier in the genus Sphingobium. The name Sphingobium aromaticiconvertens sp. nov. is proposed for this dibenzofuran-mineralizing organism, with type strain RW16T (=DSM 12677T = CIP 109198T).

Many derivatives of polycyclic aromatic compounds are considered to be xenobiotics and, therefore, to be unknown as natural products because of their structural elements, such as halonic, nitronic or sulfonic acid substituents. So-called dioxin-like compounds are also subsumed within this class of compounds. Many of the micro-organisms capable of growth on polycyclic aromatic compounds have been assigned to the family Sphingomonadaceae (Balkwill et al., 1997; Dagher et al., 1997; Fredrickson et al., 1995; Ka et al., 1994; Lloyd-Jones & Lau, 1997; Smith-Grenier & Adkins, 1996; Yabuuchi et al., 2001). Strain RW16T was previously described as a member of a defined consortium capable of biodegradation of some monochlorinated dibenzofurans (Wittich et al., 1999). The strain originated from an enrichment culture inoculated with aerobic sediment samples from the River Elbe and was assigned originally to the genus Sphingomonas (Yabuuchi et al., 1990). Based on phylogenetic, chemotaxonomic and physiological analyses, the genus Sphingomonas has been divided into four genera, Sphingomonas, Sphingobium, Novosphingobium and Sphingopyxis (Takeuchi et al., 2001). At the time of writing, the genus Sphingobium comprised ten recognized species. Recently, a new genus, Sphingosinicella (Maruyama et al., 2006), which shares the key characteristics of the genus Sphingomonas, has been described within the family Sphingomonadaceae.

Strain RW16T was originally isolated with 3-chlorodibenzofuran as the sole source of carbon and energy for growth and energy production, using a standard mineral salts medium buffered at neutral pH (Wittich et al., 1999). All target carbon sources were directly added to this medium at concentrations ranging from 1 to 5 mM. A pure culture of the bacterium was isolated after several transfers to fresh medium.

In the present study, liquid and solid LB or R2A medium were used for the purpose of culturing strain RW16T and...
Sphingobium yanoikuyae JCM 7371T for subsequent taxonomic tests. For quinone and polar lipid extraction, cells were grown in PYE medium (Busse et al., 2005). Gram staining, oxidase and catalase tests were performed by using standard laboratory procedures. Other physiological and biochemical characterizations were performed as described by Kämpfer et al. (1991, 1997). Cultures were grown aerobically at 28 °C. Cell morphology and dimensions were determined by phase-contrast microscopy.

Bacterial DNA was purified using proteinase K lysis, phenol/chloroform extractions and 2-propanol precipitation according to Wilson (1994), and purity was confirmed with caesium chloride gradient centrifugation. The G+C content of the DNA was determined as described by Johnson (1994) using ϕ phage DNA for standardization. Separation was performed on a Merck Purospher end-capped reversed-phase HPLC column of 250 × 4 mm. The mobile phase was 20 mM triethylamine phosphate in 12 % aqueous methanol (see Supplementary Fig. S1 in IJSEM Online) by using a flow rate of 1 ml min−1 at 22 °C. The G+C content was calculated from two separate hydrolysates and two independent HPLC runs. Strain RW16T had a G+C content of 60.7 mol% (individual measurements of 60.5 and 60.9 mol%), which is the lowest value hitherto reported for a strain belonging to the genus Sphingobium.

For PCR amplification of the 16S rRNA gene the DNA was obtained by boiling single colonies in 100 µl TE buffer for about 10 min at 95 °C. A nearly complete 16S rRNA gene sequence was obtained as described by Abraham et al. (1999). The reactions were evaluated on an Applied Biosystems 377 Genetic Analyzer and the final contig was assembled using the program SEQUENCER v4.0.5 (Gene Codes Corporation). The sequence was matched in BLAST (Altschul et al., 1990) against the EMBL database (Kanz et al., 2005). The sequences were aligned using CLUSTAL X software (Thompson et al., 1997) and the phylogenetic analysis was performed using MEGA 3.1 software (Kumar et al., 2004). Tree topologies were reconstructed with the neighbour-joining algorithm with 1000 bootstrap replications, according to Junca & Pieper (2004) (Fig. 1), and with the UPGMA algorithm with Kimura two-parameter model correction with the program MEGA 3.1 (Kumar et al., 2004) (see Supplementary Fig. S1 in IJSEM Online) by using sequences obtained from the EMBL database (Kanz et al., 2005). The 16S rRNA gene sequence of strain RW16T showed 94.7 % similarity to that of Sphingobium chlorophenolicum ATCC 33790T (Wittich et al., 1999), 94.9 % to Sphingobium yanoikuyae Gifu 9882T and 95.0 % (closest match found) to Sphingobium xenophagum BN6T (Pal et al., 2006). These low sequence similarities indicate that strain RW16T represents a novel species, and is closest to the genus Sphingobium in both the neighbour-joining and the UPGMA phylogenetic trees.

Bacterial polyamines were extracted and analysed by HPLC as described by Busse & Auling (1988) and Busse et al. (1997). The 16S rRNA gene signature nucleotides and the polyamine pattern of RW16T were in accordance with the characteristics of the genus Sphingobium.

Respiratory quinones were extracted and analysed by HPLC as described by Tindall (1990) and Altenburger et al. (1996). Strain RW16T contained only ubiquinone Q-10.

Polar lipids were extracted and analysed by TLC according to Tindall (1990). The polar lipid profile of RW16T is given in the species description and in Supplementary Table S1. Unlike other members of the genus Sphingobium (Busse et al., 1999), strain RW16T lacked phosphatidylcholines and phosphatidylethanolamine, and phosphatidylglycerol and phosphatidyldimethylethanolamine were detected in only minor amounts.

For structural analyses lipids were extracted by a modified Bligh–Dyer method (Fredrickson et al., 1986), followed by analysis of selected fractions in the mass spectrometer using fast atom bombardment ionization as described by Abraham et al. (1997). The polar lipid profile of strain RW16T included a high diversity of phosphatidylglycerols, many of them with the mass of fatty acid C19:1 or C19:0 cyclo (Table 1). Owing to the selective ionization mechanisms, small amounts of phosphatidylcholines not detected by TLC were seen and identified (Supplementary Table S2). In contrast to all other members of the genus Sphingobium, for strain RW16T a number of lipids were detected with masses > 1 kDa. Their fragmentation patterns did not match with known diphasphatidylglycerols and their structures could not be determined as a result of their low abundances.

**Fig. 1.** Unrooted neighbour-joining dendrogram of the phylogenetic relationships between strain RW16T, Sphingobium species, Sphingomonas cloacae and the type species of the genera Sphingomonas, Sphingopyxis, Novosphingobium and Sphingosinicella based on a distance matrix analysis of 16S rRNA gene sequences. Accession numbers are given in parentheses. Bootstrap percentages are indicated at tree branching points. Bar, 0.01 substitutions per nucleotide position.
Table 1. Cellular fatty acid composition of strain RW16T, Sphingobium species and Sphingomonas claoae

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1 2 3 4 5 6 7 8 9 10 11</th>
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<tr>
<td>C14:0</td>
<td>0.5 0.1 0.2 0.2 1.2</td>
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<tr>
<td>C16:0</td>
<td>8 9.5 12 7.2 16.5 7.4 19.0 10.0 7.8 8.0 11.2</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.3</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.7 0.4 0.9 0.9</td>
</tr>
<tr>
<td>C16:1ω5c</td>
<td>2.4 1.3 3.3 1.2 1.6 0.9 2.2 2.0 2.7</td>
</tr>
<tr>
<td>C16:1ω9c</td>
<td>0.7</td>
</tr>
<tr>
<td>C17:1ω6c</td>
<td>2 6.4 2 1.7 2.5 3.2</td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>2 5.3 1.3 1.1 2.0 1.6 1.4 1.6 2.1</td>
</tr>
<tr>
<td>C12:0 ω2-OH</td>
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<td>C13:0 ω2-OH</td>
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<tr>
<td>C14:0 ω2-OH</td>
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</tr>
<tr>
<td>C16:0 ω2-OH</td>
<td>0.2 0.2 0.2 0.2 0.2 0.2 0.9</td>
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Summed feature 4 consists of C16:1ω7c and/or iso-C15:0 2-OH. Summed feature 7 consists of one or more of C18:1ω7c, C18:1ω9t, and C18:1ω12t.

16S rRNA gene sequence similarities as well as phylogenetic calculations demonstrated that strain RW16T can be placed distantly from recognized species of the family Sphingomonadaceae. Signature nucleotides characteristic for the genus Sphingobium as reported by Takeuchi et al. (2001) were found in the 16S rRNA gene sequence of strain RW16T. To find additional support for the generic placement of strain RW16T, its cellular fatty acids were compared with those of key species of genera of the Sphingomonadaceae. GC was used to analyse the fatty acid profiles of strain RW16T grown on R2A agar, as described by Kämpfer et al. (1992). Strain RW16T possessed the fatty acid C17:0 cyclo, which is atypical of members of the Sphingomonadaceae (Busse et al., 1999) (Table 1). The five genera of the family Sphingomonadaceae have the same dominant fatty acids (C18:1ω7c and C16:1ω7c), and the genus Sphingopyxis differs only in lacking C14:0 2-OH. Therefore, cellular fatty acids may not offer additional information to define the generic placement of strain RW16T. Strain RW16T was positive for β-galactosidase, as is the type species of the genus Sphingobium but in contrast to many other Sphingobium species (Table 2). The 16S rRNA gene sequence signature nucleotides and the polyamine pattern placed strain RW16T within the genus Sphingobium, where it can be fitted to a deep branch. Strain RW16T is thus suggested to represent a new species of the genus Sphingobium, for which the name Sphingobium aromaticiconvertens sp. nov. is proposed.

Table 2. Differential biochemical characteristics of strain RW16T, Sphingobium species and Sphingomonas claoae

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tr>
<td>d-Glucose</td>
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<td>−</td>
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<td>+</td>
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<td>+</td>
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<td>−</td>
<td>ND</td>
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</tbody>
</table>
D-melibiose, sucrose, salicin, D-trehalose, D-xylene, maltitol, D-mannitol, D-sorbitol, acetate, propionate, cis-aconitate, adipate, 4-aminobutyrate, azelate, citrate, fumarate, DL-3-hydroxybutyrate, L-malate, pyruvate, L-alanine, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. p-Nitrophenyl (pNP) α-D-glucopyranoside, bis-pNP phosphate and L-alanine p-nitroanilide (pNA) are hydrolysed, but aesculin, pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP β-D-glucopyranoside, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxymyridine-5′-pNP phosphate, L-glutamate-3-carboxy pNA and L-proline pNA are not. In the polar lipid profile, diphasatidyglycerol, sphingoglycolipid and an unknown glycolipid are predominant. Phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerolphosphate and an unknown glycolipid are present in minor amounts. Major fatty acids are C18:1ω9 (included within summed feature 7) and C14:0 2-OH and minor fatty acids are C16:0, C18:1ω6c and C14:0. Major cellular polyamine is spermidine (30.2 μmol g⁻¹ dry weight); minor amounts of spermine (2.7 μmol g⁻¹ dry weight) and traces of putrescine and cadaverine are present as well. The quinone system is characterized by ubiquinone Q-10 only. The DNA G+C content is 60.7 mol%.

The type strain, RW16T (=DSM 12677T=CIP 109198T), was isolated from an enrichment culture on some monochlorinated dibenzofurans inoculated with aerobic sediment samples from the River Elbe, Germany.

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References


