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 Tiirola,5 Monika Wieser,3 Alexandre J. Macedo1 and Wolf-Rainer Abraham1

Correspondence
Wolf-Rainer Abraham
wab@gbf.de

1Helmholtz Zentrum für Infektionsforschung (formerly GFB), 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 (1974). The 16S rRNA gene sequence of strain RW16T was obtained from the EMBL database (Kanz et al., 2005) (see Supplementary Fig. S1 in IJSEM Online) by using λ 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 at 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 with the programMEGA 3.1 (Kumar et al., 2001) 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 chlorophenicolicum 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.

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 lacked phosphatidylcholine and phosphatidylethanolamine, and phosphatidylglycerol and phosphatidylglycerolphosphate were detected in only minor amounts.

Fig. 1. Unrooted neighbour-joining dendrogram of the phylogenetic relationships between strain RW16T, Sphingobium species, Sphingomonas clocaceae 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.

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 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 phosphatidylethanolamines 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 diphosphatidylglycerols and their structures could not be determined as a result of their low abundances.
16S rRNA gene sequence similarities as well as phylogenetic calculations demonstrated that strain RW16\textsuperscript{T} 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 RW16\textsuperscript{T}. To find additional support for the generic placement of strain RW16\textsuperscript{T}, its cellular fatty acids were compared with the type species of genera of the Sphingomonadaceae. GC content was used to analyze the fatty acid profiles of strain RW16\textsuperscript{T} grown on R2A agar, as described by Kämpfer et al. (1992). Strain RW16\textsuperscript{T} possessed the fatty acid C\textsubscript{17:0} 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 (C\textsubscript{18:1} and C\textsubscript{16:0}), and the genus Sphingopyxis differs only in lacking C\textsubscript{14:0} 2-OH.

Therefore, cellular fatty acids may not offer additional information to define the generic placement of strain RW16\textsuperscript{T}. Strain RW16\textsuperscript{T} was positive for \(\beta\)-galactosidase, as is the type species of the genus Sphingobium but in contrast to many other Sphingobium species (Table 2). The 16S rRNA gene signature nucleotides and the polyamine pattern placed strain RW16\textsuperscript{T} within the genus Sphingobium, where it can be fitted to a deep branch. Strain RW16\textsuperscript{T} is thus suggested to represent a novel species of the genus Sphingobium, for which the name Sphingobium aromaticiconvertens sp. nov. is proposed.

### Description of Sphingobium aromaticiconvertens sp. nov.

Sphingobium aromaticiconvertens \[a.ro.ma.ti'ci.con.ver'tens.\] L. adj. \(\textit{aromaticus}\) aromatic; N.L. neut. \(\textit{aromaticum}\) aromatic compound; L. part. adj. \(\textit{convertens}\) converting; N.L. part. adj. \(\textit{aromaticiconvertens}\) converting aromatic compounds (into other form).

The description is the same as that given for the genus with the following additional characteristics. Colonies are light yellow on mineral salts medium and on LB medium. Cells are short, irregular rods, 0.8–1.5 \(\mu\)m in length and 0.4–0.6 \(\mu\)m in diameter, forming rosettes in complex medium; on selective media, cells form branched thread-like/hypha-like aggregates. A large capsule is formed during growth with dibenzoferan. Gram-negative and oxidase- and catalase-positive. Nitrate is not reduced to nitrite. D-Fructose, L-rhamnose, D-lactate and l-aspartate are used as substrates for growth but not N-acetyl-D-glucosamine, L-arabinose, p-aminobenzoic, D-cellobiose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose.

### Table 1. Cellular fatty acid composition of strain RW16\textsuperscript{T}, Sphingobium species and Sphingomonas clacetae

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>C\textsubscript{14:0}</td>
<td>0.5</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{16:0}</td>
<td>8</td>
<td>9.5</td>
<td>12</td>
<td>7.2</td>
<td>16.5</td>
<td>7.4</td>
<td>19.0</td>
<td>10.0</td>
<td>7.8</td>
<td>8.0</td>
<td>11.2</td>
</tr>
<tr>
<td>C\textsubscript{17:0}</td>
<td>0</td>
<td>0.4</td>
<td>0.9</td>
<td>0.4</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{18:1} (v)</td>
<td>2.4</td>
<td>3.3</td>
<td>1.2</td>
<td>1.6</td>
<td>0.9</td>
<td>2.2</td>
<td>2.0</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{18:1} (c)</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{17:0} (c)</td>
<td>2</td>
<td>6.4</td>
<td>2</td>
<td>1.7</td>
<td>2.5</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{18:1} (c)</td>
<td>2.5</td>
<td>3</td>
<td>1.3</td>
<td>1.1</td>
<td>2.0</td>
<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{12:0} 2-OH</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{13:0} 2-OH</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{14:0} 2-OH</td>
<td>7</td>
<td>9.4</td>
<td>2.8</td>
<td>5.1</td>
<td>5.5</td>
<td>6.8</td>
<td>4.3</td>
<td>11.8</td>
<td>6.7</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>C\textsubscript{16:0} 2-OH</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 4*</td>
<td>13</td>
<td>9.6</td>
<td>6</td>
<td>13.4</td>
<td>0.2</td>
<td></td>
<td></td>
<td>23.9</td>
<td>16.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 7*</td>
<td>62</td>
<td>60.1</td>
<td>67</td>
<td>80</td>
<td>62.5</td>
<td>68.3</td>
<td>55.7</td>
<td>66.7</td>
<td>74.8</td>
<td>55.2</td>
<td>56.0</td>
</tr>
</tbody>
</table>

*Summed feature 4 consists of C\textsubscript{16:1} \(v\) \(t\) and/or iso-C\textsubscript{15:0} 2-OH. Summed feature 7 consists of one or more of C\textsubscript{18:1} \(v\) \(t\), C\textsubscript{18:1} \(o\) \(9t\) and C\textsubscript{18:1} \(o\) 12t.

### Table 2. Differential biochemical characteristics of strain RW16\textsuperscript{T}, Sphingobium species and Sphingomonas clacetae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate to nitrite</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\beta)-Galactosidase</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1. Sphingobium amience ICM 11777\textsuperscript{T} (data from Ushiba et al., 2003); 2. Sphingobium chlorophenolicum ATCC 33790\textsuperscript{T} (data from this study); 3. Sphingobium chungbukense D777\textsuperscript{T} (Kim et al., 2000); 4. Sphingobium clacetae ICM 10874\textsuperscript{T} (Fujii et al., 2001); 5. Sphingobium francense CCM 7288\textsuperscript{T} (Pal et al., 2005); 6. Sphingobium herbicidovorans DSM 11019\textsuperscript{T} (this study); 7. Sphingobium indicum CCM 7286\textsuperscript{T} (Pal et al., 2005); 8. Sphingobium japonicum CCM 7287\textsuperscript{T} (Pal et al., 2005); 9. strain RW16\textsuperscript{T}; 10. Sphingobium xenaphagum DSM 6383\textsuperscript{T}; 11. Sphingobium yanoikuyae IFO 15102\textsuperscript{T} (data in columns 9–11 from this study). Values shown are percentages of total fatty acids.

2. Sphingobium amience YT\textsuperscript{T}; 3. Sphingomonas clacetae ICM 10874\textsuperscript{T}; 4. Sphingobium yanoikuyae ICM 7371\textsuperscript{T}; 5. Sphingobium chlorophenolicum DSM 8671\textsuperscript{T}; 6. Sphingobium herbicidovorans DSM 11019\textsuperscript{T}; 7. Sphingobium chungbukense KCTC 2955\textsuperscript{T}; 8. Sphingobium indicum B90A\textsuperscript{T}; 9. Sphingobium japonicum UT26\textsuperscript{T}; 10. Sphingobium francense SP +\textsuperscript{T}; 11. Sphingobium xenaphagum BN6\textsuperscript{T}. Data in columns 2–6 are from Ushiba et al. (2003), in column 7 from Kim et al. (2000), in columns 8–10 from Pal et al. (2005) and in column 11 from Stolz et al. (2000). Symbols: +, positive; −, negative; ND, no data available.
D-melibiose, sucrose, salicin, D- trehalose, D-xylose, 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 acetyl, pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP β-D-glucopyranoside, pNP phenylphosphonate, pNP phosphorolylcholine, 2-deoxymyridine-5′-pNP phosphate, L-glutamate-3-carboxy pNA and L-proline pNA are not. In the polar lipid profile, diphasitidyglycerol, sphingoglycolipid and an unknown glycolipid are predominant. Phosphatidylinositolmonophethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid are present in minor amounts. Major fatty acids are C₁₈:1 (included within summed feature 7) and C₁₄:2-OH and minor fatty acids are C₁₆:0, C₁₈:1ω₇c and C₁₄:1ω6c. 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.

Acknowledgements

The excellent assistance of Jennifer Skerra is gratefully acknowledged. The GBF work was supported in part by grant BMGF 0319378B. A. J. M. acknowledges support in the form of a grant of the German Academic Exchange Service (DAAD). We thank Norbert Weiss and Susanne Toepffer for their help with the use of Latin for nomenclatural purposes.

References


