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

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A bacterial strain capable of degrading some monochlorinated dibenzofurans, designated RW16^T, 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 RW16^T represents a novel species of the genus *Sphingobium*. The DNA G + C content of strain RW16^T, 60.7 mol%, is the lowest yet reported for the genus. 16S rRNA gene sequence analysis placed strain RW16^T as an outlier in the genus *Sphingobium*. The name *Sphingobium aromaticiconvertens* sp. nov. is proposed for this dibenzofuran-mineralizing organism, with type strain RW16^T (=DSM 12677^T = CIP 109198^T).

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 RW16^T 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 RW16^T 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 RW16^T and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RW16^T is AM181012.

A tree generated by UPGMA and details of the distribution of polar lipids in strain RW16^T and related species are available as supplementary material in IJSEM Online.

Sphingobium yanoikuyae JCM 7371^T 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 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 RW16^T 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 μ l 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 SEQUENCHER v4.0.5 (Gene Codes Corporation). The sequence was matched in BLAST 2.2.9 (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 RW16^T showed 94.7 % similarity to that of *Sphingobium chlorophenolicum* ATCC 33790^T (Wittich *et al.*, 1999), 94.9 % to *Sphingobium yanoikuyae* GIFU 9882^T and 95.0 % (closest match found) to *Sphingobium xenophagum* BN6^T (Pal *et al.*, 2006). These low sequence similarities indicate that strain RW16^T 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

Fig. 1. Unrooted neighbour-joining dendrogram of the phylogenetic relationships between strain RW16^T, *Sphingobium* species, *Sphingomonas cloacae* and the type species of the genera *Sphingomonas*, *Sphingopyxis*, *Novosphingobium* and *Sphingosinella* 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.

polyamine pattern of RW16^T 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 RW16^T contained only ubiquinone Q-10.

Polar lipids were extracted and analysed by TLC according to Tindall (1990). The polar lipid profile of RW16^T is given in the species description and in Supplementary Table S1. Unlike other members of the genus *Sphingobium* (Busse *et al.*, 1999), strain RW16^T lacked phosphatidylcholine and phosphatidylethanolamine, and phosphatidylglycerol and phosphatidylmethylethanolamine 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 RW16^T included a high diversity of phosphatidylglycerols, many of them with the mass of fatty acid C_{19:1} or C_{19:0} 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 RW16^T 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.

Table 1. Cellular fatty acid composition of strain RW16^T, *Sphingobium* species and *Sphingomonas cloacae*

Strains: 1, *Sphingobium amiense* JCM 11777^T (data from Ushiba *et al.*, 2003); 2, *Sphingobium chlorophenolicum* ATCC 33790^T (data from this study); 3, *Sphingobium chungbukense* DJ77^T (Kim *et al.*, 2000); 4, *Sphingomonas cloacae* JCM 10874^T (Fujii *et al.*, 2001); 5, *Sphingobium francense* CCM 7288^T (Pal *et al.*, 2005); 6, *Sphingobium herbicidovorans* DSM 11019^T (this study); 7, *Sphingobium indicum* CCM 7286^T (Pal *et al.*, 2005); 8, *Sphingobium japonicum* CCM 7287^T (Pal *et al.*, 2005); 9, strain RW16^T; 10, *Sphingobium xenophagum* DSM 6383^T; 11, *Sphingobium yanoikuyae* IFO 15102^T (data in columns 9–11 from this study). Values shown are percentages of total fatty acids.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
C _{14:0}		0.5		0.1	0.2			0.2	1.2		
C _{16:0}	8	9.5	12	7.2	16.5	7.4	19.0	10.0	7.8	8.0	11.2
C _{17:0}				0.3							
C _{18:0}				0.7	0.4		0.9				
C _{16:1} ω5c		2.4	1	3.3	1.2	1.6	0.9	2.2		2.0	2.7
C _{16:1} ω9c				0.7							
C _{17:1} ω6c	2	6.4	2			1.7				2.5	3.2
C _{17:0} cyc7,8									2.6		
C _{18:1} ω5c		2.5	3	1.3	1.1	2.0	1.6	1.4	1.6	1.6	2.1
C _{12:0} 2-OH						1.0					
C _{13:0} 2-OH						2.6					
C _{14:0} 2-OH	7	9.4	9	2.8	5.1	5.5	6.8	4.3	11.8	6.7	7.1
C _{16:0} 2-OH				0.2	0.2		0.2				0.9
Summed feature 4*	13	9.6	6			13.4	0.2			23.9	16.3
Summed feature 7*	62	60.1	67	80	62.5	68.3	55.7	66.7	74.8	55.2	56.0

*Summed feature 4 consists of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH. Summed feature 7 consists of one or more of C_{18:1}ω7c, C_{18:1}ω9t and C_{18:1}ω12t.

16S rRNA gene sequence similarities as well as phylogenetic calculations demonstrated that strain RW16^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^T. To find additional support for the generic placement of strain RW16^T, its cellular fatty acids were compared with the type species of genera of the *Sphingomonadaceae*. GC was used to analyse the fatty acid profiles of strain RW16^T grown on R2A agar, as described by Kämpfer *et al.* (1992). Strain RW16^T possessed the fatty acid C_{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_{18:1} and C_{16:0}), and the genus *Sphingopyxis* differs only in lacking C_{14:0} 2-OH. Therefore, cellular fatty acids may not offer additional information to define the generic placement of strain RW16^T. Strain RW16^T 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 signature nucleotides and the polyamine pattern placed strain RW16^T within the genus *Sphingobium*, where it can be fitted to a deep branch. Strain RW16^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. *aromaticus* aromatic; N.L. neut. n. *aromaticum* aromatic compound; L. part. adj. *convertens* converting; N.L. part. adj. *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 µm in length and 0.4–0.6 µm 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 dibenzofuran. Gram-negative and oxidase-, β-galactosidase- and catalase-positive. Nitrate is not reduced to nitrite. D-Fructose, L-rhamnose, DL-lactate and L-aspartate are used as substrates for growth but not N-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose,

Table 2. Differential biochemical characteristics of strain RW16^T, *Sphingobium* species and *Sphingomonas cloacae*

Strains: 1, RW16^T; 2, *Sphingobium amiense* YT^T; 3, *Sphingomonas cloacae* JCM 10874^T; 4, *Sphingobium yanoikuyae* JCM 7371^T; 5, *Sphingobium chlorophenolicum* DSM 8671^T; 6, *Sphingobium herbicidovorans* DSM 11019^T; 7, *Sphingobium chungbukense* KCTC 2955^T; 8, *Sphingobium indicum* B90A^T; 9, *Sphingobium japonicum* UT26^T; 10, *Sphingobium francense* SP+^T; 11, *Sphingobium xenophagum* BN6^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.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Assimilation of:											
D-Glucose	–	+	–	+	+	+	+	+	+	+	+
L-Arabinose	–	–	–	+	–	+	+	+	+	+	+
Fructose	+	ND	–	–	–	–	+	+	+	–	–
D-Mannose	–	–	–	–	–	–	+	–	–	–	–
N-Acetyl-D-glucosamine	–	–	–	+	–	–	–	ND	ND	ND	–
Maltose	–	+	–	+	–	+	+	ND	ND	ND	+
Aesculin hydrolysis	–	–	–	+	+	+	–	–	+	+	+
Nitrate to nitrite	–	–	–	–	–	–	–	ND	ND	ND	ND
β-Galactosidase	+	–	–	+	–	–	–	ND	ND	ND	–

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 aesculin, pNP β -D-galactopyranoside, pNP β -D-glucuronide, pNP β -D-glucopyranoside, pNP phenylphosphonate, pNP phosphorylcholine, 2-deoxythymidine-5'-pNP phosphate, L-glutamate-3-carboxy pNA and L-proline pNA are not. In the polar lipid profile, diphosphatidylglycerol, sphingoglycolipid and an unknown glycolipid are predominant. Phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmethylethanolamine, an unknown phospholipid and an unknown glycolipid are present in minor amounts. Major fatty acids are C_{18:1} (included within summed feature 7) and C_{14:0} 2-OH and minor fatty acids are C_{16:0}, C_{18:1} ω 5c and C_{14:0}. Major cellular polyamine is spermidine (30.2 μ mol g⁻¹ dry weight); minor amounts of spermine (2.7 μ mol 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, RW16^T (=DSM 12677^T=CIP 109198^T), was isolated from an enrichment culture on some monochlorinated dibenzofurans inoculated with aerobic sediment samples from the River Elbe, Germany.

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