Wittich, R.-M., Busse, H.-J., Kämpfer, P., Tiirola, M., Wieser, M., Macedo, A.J., Abraham, W.-R.

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

(2007) International Journal of Systematic and Evolutionary Microbiology, 57 (2), pp. 306-310.

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

Rolf-Michael Wittich,[1,5] Hans-Jürgen Busse,[2] Peter Kämpfer,[3] Marja Tiirola,[4] Monika Wieser,[2] Alexandre J. Macedo[1] and Wolf-Rainer Abraham[1*]

[1]Helmholtz Zentrum für Infektionsforschung (früher GBF), Division Microbiology, D-38124 Braunschweig, Germany,

[2]Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, A-1210 Wien, Austria,

[3]Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität, D-35390 Giessen, Germany,

[4]Department of Biological and Environmental Science, University of Jyväskylä, FI-40100 Jyväskylä, Finland,

[5]Estación Experimental del Zaidín - EEZ-CSIC, Depto. de Bioquímica, Biología Celular y Molecular, Línea de Degradación de Tóxicos Orgánicos, Calle Profesor Albareda 1 E - 18008 Granada, Spain,

Running title: *Sphingobium aromaticiconvertens* sp. nov.

Key words: *Sphingobium aromaticiconvertens* sp. nov., biodegradation, xenobiotics, polyphasic taxonomy, polar lipids, polyamines

* Author for correspondence:
Dr. Wolf-Rainer Abraham

Helmholtz Institute for Infection Research (former GBF)
Chemische Mikrobiologie
Inhoffenstrasse 7
38124 Braunschweig
Germany

Phone: *49-531-6181 4300
Fax: *49-531-6181 4699
E-mail: wab@gbf.de

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

Summary

Strain RW16^T isolated from aerobic River Elbe sediments is capable to degrade some monochlorinated dibenzofurans. The strain was characterized by 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 is 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 places strain RW16^T as an outlier in the genus *Sphingobium*. We propose the dibenzofuran mineralising species *Sphingobium aromaticiconvertens* sp. nov. with the type strain RW16^T (DSM 12677^T, CIP 109198^T).

Many derivatives of polycyclic aromatic compounds are considered as xenobiotics and, therefore, unknown as natural products because of their structural elements, such as halo, nitro or sulfonic acid substituents. So-called dioxin-like compounds are also subsumed to this class of compounds. Many of the isolates growing on polycyclic aromatic compounds have been assigned to the family *Sphingomonadaceae* (Balkwil *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 (DSM 12677^T) was 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 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, Sphingopyxis* (Takeuchi *et al.* 2001) and *Sphingosinicella* (Maruyama et al., 2006). The genus *Sphingobium* comprises currently ten species Recently, a novel genus, *Sphingosinicella* (Maruyama *et al.*, 2006), which shares the key characteristics of the genus *Sphingomonas,* has been described within the family.

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 corresponding to one up to 5.0 mM. A pure culture of the bacterium was isolated after several transfers to fresh medium.

In this study liquid and solid LB or R2A medium were used for the purpose of culturing RW16^T and *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 made by standard laboratory procedures.

Other physiological and biochemical characterizations were made as described earlier (Kämpfer *et al.,* 1997 and 1991). Cultures were grown aerobically at 28°C. Cell morphology and dimensions were determined by phase-contrast microscopical investigations.

Bacterial DNA was purified using proteinase K lysis, phenol-chloroform extractions, and isopropanol precipitation according to Wilson (1994), and the purity was confirmed with cesium chloride gradient centrifugation. G+C % content was determined as described by Johnson (1994) using λ phage DNA for standardization. The separation was performed on a Merck Purospher endcapped reversed phase HPLC column of 250 by 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 ± 0.2% , which is the lowest value hitherto reported for a strain of this genus.

For the amplification of the 16S rRNA gene by polymerase chain reaction the DNA was obtained by boiling single colonies in 100 µl of TE buffer for about 10 min at 95°C. A nearly complete 16S rRNA gene sequence was obtained as described previously (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™ Version 4.0.5 (Gene Codes Corporation, USA). 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 neighbour-joining algorithm with 1000 bootstrap replications, according to Junca and Pieper (2004) (Fig. 1), and the UPGMA algorithm with Kimura-2 parameter was calculated with the software MEGA 3.1. (Kumar *et. Al.*, 2004) (IJSEM Online Supplementary figure A) using the sequences obtained from the EMBL database (Kanz *et al*., 2005). The 16S rRNA gene sequence of strain

RW16^T showed 94.7 % similarity to the 16S rDNA sequence of *Sphingobium chlorophenolicum* ATCC 33790^T (Wittich *et al*., 1999), 94.9% to *Sphingobium yanoikuyae* JCM 7371^T and 95.0% to the closest established species, *Sphingobium xenophagum* DSM 6383^T (Pal *et al*., 2006). These low sequence similarities qualify strain RW16^T as a novel species which is closest to the genus *Sphingobium* in both the 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 RW16^T were in accordance with the characteristics of the genus *Sphingobium*.

Respiratory quinones were extracted and analysed by HPLC as previously described (Tindall, 1990; 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 listed in the species description and Table 3 (IJSEM supplementary data). Unlike other species of the genus *Sphingobium* (Busse *et al*., 1999) RW16^T lacked phosphatidylcholine and phosphatidylethanolamine, phosphatidylglycerol and phosphatidyldimethylethanolamine were detected in 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 (FAB-MS) ionisation as previously described by Abraham *et al.* (1997). The polar lipids found in strain RW16^T displayed a high diversity of phosphatidylglycerols many of them with the mass of fatty acid $C_{19:1}$ or cyclo $C_{19:0}$ (Table 2). Due to the selective ionisation mechanisms small amounts of phosphatidylcholines not detected by TLC were seen and identified (Table 3). Contrary to all other *Sphingobium* strains a number of lipids were detected with masses >1000 Da. Their fragmentation pattern did not match with known diphosphatidylglycerols and their structures could not be determined due to their low abundances.

16S rRNA gene sequence similarities as well as phylogenetic calculations demonstrated that strain RW16^T is relatively far from other known 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 genus harbouring strain RW16^T its cellular fatty acids were compared with type species of the *Sphingomonadaceae*. Gas chromatography (GC) was used to analyse the fatty acid profiles of the strain grown on R2A agar, as described previously (Kämpfer *et al*., 1992). Strain RW16^T possessed the fatty acid 17:0 cyclo, which is highly untypical for *Sphingomonadaceae* (Busse *et al*., 1999) (Table 2). All the four genera of the family *Sphingomonadaceae* have the same dominant fatty acids ($C_{18:1}$ and $C_{16:0}$) and 2-OH $C_{14:0}$ was the only hydroxy fatty acid which is different from *Sphingopyxis.* Therefore, cellular fatty acids may not offer additional information for defining the right genus for RW16^T. The strain was β-galactosidase positive as is the type species of *Sphingobium* but contrary to many other *Sphingobium* species. The 16S rRNA gene signature nucleotides and the polyamine pattern accommodated strain RW16^T to the genus *Sphingobium*, where it can be fitted to a deep branch of the genus. Hence, we propose to place this species into the genus *Sphingobium* as *Sphingobium aromaticiconvertens* sp. nov.

Proposal of novel species

Description of *Sphingobium aromaticiconvertens* sp. nov.

The description of *Sphingobium aromaticiconvertens* (aro.ma.ti.ci.con.ver'tans. Chem. n. *aromatic*, aromatic compound; L. v. convertere, transform; *convertans*, transforming; *aromaticiconvertens*, converting aromatic compounds into other form) is the same as that given for the genus with the following additional characteristics. Colonies colored lightly

yellow on mineral salts medium and on LB media. Cells of this species are short clumsy rods, 0.8 - 1.5 μm in length and 0.4 - 0.6 μm in diameter, forming rosettes in complex medium and when grown on selective media the cells tended to grow as branched thread-like/hyphae-like aggregates.

A large capsule is formed during growth with dibenzofuran. Gram stain is negative, oxidase, β-galactosidase and catalase positive. Nitrate is not reduced to nitrite. D-fructose, L-rhamnose, DL-lactate, and L-aspartate are used as substrate but not N-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose,. D-melibiose, sucrose, salicin, D-trehalose, D-xylose, maltitol, D-mannitol, D-sorbitol, acetate, propinate, *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-tryptophane, 3-hydroxybenzoate, 4-hydroxybenzoate, phenylacetate. pNP-a-D-glucopyranoside, bis-pNP-phosphate and L-alanine-pNA are hydrolysed, but not esculin, pNP-ß-D-galactopyranoside, pNP-ß-D-glucuronide, pNP-ß-D-glucopyranoside, pNP-phenyl phosphonate, pNP-phosphoryl choline, 2-deoxythymidine-5'-pNP-phosphate, L-glutamate-3-carboxy-pNA, L-proline-pNA.

In the polar lipid profile diphosphatidylglycerol, sphingoglycolipid and an unknown glycolipid are predominant. Phosphatidylmonomethylethanolamine, phosphatidylethanol-amine, phosphatidylglycerol, phosphatidyldimethylethanolamine, an unknown phospholipid and an unknown glycolipid are present in minor amounts. The species is characterized by the major fatty acids $C_{18:1}$ (Sum7) and 2-hydroxy-$C_{14:0}$ and the minor fatty acids $C_{16:0}$, $C_{18:1}\omega5c$ and $C_{14:0}$. 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 characterised by ubiquinone Q-10 only; the G+C content is 60.7 mol%. Isolated from an enrichment culture on some monochlorinated dibenzofurans

inoculated with aerobic sediment samples from the river Elbe, Germany. The type strain is RW16^T (= DSM 12677^T, = CIP 109198^T).

ACKNOWLEDGEMENTS

The excellent assistence of Jennifer Skerra is gratefully acknowledged. The GBF work was supported in part by grant BMBF 0319378B. AJM acknowledges the support by 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

Abraham, W.-R., Meyer, H., Lindholst, S., Vancanneyt, M. & Smit, J. (1997). Phospho- and sulfolipids as biomarkers of *Caulobacter sensu lato*, *Brevundimonas* and *Hyphomonas*. *System Appl Microbiol* **20**, 522-539.

Abraham, W.-R., Stroempl, C., Meyer, H., Lindholst, S., Moore, E. R. B., Christ, R., Vancanneyt, M., Tindall, B., Bennasar, A., Smit, J. & Tesar, M. (1999). Phylogeny and polyphasic taxonomy of *Caulobacter* species. Proposal of *Maricaulis* gen. nov. with *Maricaulis maris* (Poindexter) comb. nov. as the type species, and emended description of the genera *Brevundimonas* and *Caulobacter*. *Int J Syst Bacteriol* **49**, 1053-1073.

Altenburger, P., Kämpfer, P., Makristathis A., Lubitz, W. & Busse H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39-52.

Altschul, S. F., Gish, W., Miller, W., Meyers, E.W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403-410.

Balkwil, D. L., Drake, G. R., Reeves, R. H., Fredrickson, J. K., White, D. C., Ringelberg, D. B., Chandler, D. P., Romine, M. F., Kennedy, D. E. & Spadoni, C. M. (1997). Taxonomic study of aromatics-degrading bacteria from the deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *Int J Syst Bacteriol* **47**, 191-201.

Busse, H.-J., & Auling, G. (1988). Polyamine patterns as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1-8.

Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698-708.

Busse, H.-J., Hauser, E. & Kämpfer, P. (2005). Description of two novel species, *Sphingomonas abaci* sp. nov. and *Sphingomonas panni* sp. nov. *Int J Syst Evol Microbiol* **55**, 2565-2569.

Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterisation of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242-251.

Dagher, F., Deziel, E., Lirette, P., Paquette, G., Bisaillon, J. G. & Villemur, R. (1997). Comparative study of five polycyclic aromatic hydrocarbon degrading bacterial strains isolated from contaminated soils. *Can J Microbiol* **43**, 368-377.

Fredrickson, H. L., Cappenberg, T. E. & De Leeuw, J. (1986). Polar lipid fatty acid composition of Lake Vechten seston - an ecological application of lipid analysis. *FEMS Microbiol Ecol* **38**, 381-396.

Fredrickson, J. K., Balkwill, D. L., Drake, G. R., Romine, M. F., Ringelberg, M. F. & White, D. C. (1995). Aromatic-degrading *Sphingomonas* isolates from the deep subsurface. *Appl Environ Microbiol* **61**, 1917-1922.

Fujii, K., Urano, N., Ushio, H., Satomi, M. & Kimura, S. (2001) *Sphingomonas cloacae* sp. nov., a nonylphenoldegrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo. *Int J Syst Evol Microbiol* **51**, 603-610.

Johnson, J. L. (1994). Similarity analysis of DNAs, In *Methods for General and Molecular Bacteriology,* pp. 664-666. Edited by P. Gerhardt, R.G.E. Murray, W.A. Wood & N.R. Krieg. Washington, D.C.: American Society for Microbiology.

Junca, H., & Pieper, D. H. (2004). Functional gene diversity analysis in BTEX contaminated soils by means of PCR-SSCP DNA fingerprinting: comparative diversity assessment against bacterial isolates and PCR-DNA clone libraries. *Environ Microbiol* **6,** 95-110.

Ka, J. O., Holben, W. E. & Tiedje, J . M. (1994). Genetic and phenotypic diversity of 2,4-dichlorophenoxyacetic acid (2,4-D)-degrading bacteria isolated from 2,4-D- treated field soils. *Appl Environ Microbiol* **60**, 1106-1115.

Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227-251.

Kämpfer, P., Bark, K., Busse, H.-J., Auling, G. & Dott, W. (1992). Numerical and chemotaxonomy of polyphosphate accumulating *Acinetobacter* strains with high polyphosphate: AMP phosphotransferase (PPAT) activity. *System Appl Microbiol* **15**, 409-419.

Kämpfer, P., Denner, E. B. M., Meyer, S., Moore, E. R. B. & Busse, H.-J. (1997). Classification of "*Pseudomonas azotocolligans*" Anderson 1955, 132, in the genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov. *Int J Syst Bacteriol* **47**, 577-583.

Kanz, C., Aldebert, P., Althorpe, N., Baker,W., Baldwin, A., Bates, K., Browne, P., van den Broek, A., Castro, M., Cochrane, G., Duggan, K., Eberhardt, R., Faruque, N., Gamble, J., Diez, F.G., Harte, N., Kulikova, T., Lin, Q., Lombard, V., Lopez, R., Mancuso, R., McHale, M., Nardone, F., Silventoinen, V., Sobhany, S., Stoehr, P., Tuli, M. A., Tzouvara, K., Vaughan, R., Wu, D., Zhu, W. & Apweiler, R. (2005). The EMBL nucleotide sequence database. *Nucl Acids Res* **33**, D29-D33.

Kim, S.-J., Chun, J., Bae, K. S., & Kim, Y.-C. (2000) Polyphasic assignment of an aromatic degrading *Pseudomonas* sp. strain DJ77, in the genus *Sphingomonas* as *Sphingomonas chungbukensis* sp. nov. *Int J Syst Evol Microbiol* **50**, 1641-1647.

Kumar, S., Tamura, K., & Nei, M. (2004) MEGA3: Integrated software for Molecular

Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5,**150-163.

Lloyd-Jones, G. & Lau, P. C. (1997). Glutathione S-transferase-encoding gene as a potential probe for environmental bacterial isolates capable of degrading polycyclic aromatic hydrocarbons. *Appl Environ Microbiol* **63**, 3286-3290.

Maruyama, T., Park, H. D., Ozawa, K., Tanaka, Y., Sumino, T., Hamana, K., Hiraishi, A. & Kato, K. (2006). *Sphingosinicella microcystinivorans* gen. nov., sp. nov., a microcystin-degrading bacterium. *Int J Syst Evol Microbiol* **56**, 85-89.

Pal, R., Bala, S., Dadhwal, M., Kumar, M., Dhingra, G., Prakash, O., Prabagaran, S. R., Shivaji, S., Cullum, J., Holliger, C. & Lal, R. (2005) Hexachlorocyclohexane-degrading bacterial strains *Sphingomonas paucimobilis* B90A, UT26 and Sp+, having similar lin genes, represent three distinct species, *Sphingobium indicum* sp. nov., *Sphingobium japonicum* sp. nov. and *Sphingobium francense* sp. nov., and reclassification of [*Sphingomonas*] *chungbukensis* as *Sphingobium chungbukense* comb. nov. *Int J Syst Evol Microbiol* **55**, 1965-1972.

Pal, R., Bhasin, V. K. & Lal R. (2006) Proposal to reclassify [*Sphingomonas*] *xenophaga* Stolz *et al*. 2000 and [*Sphingomonas*] *taejonensis* Lee *et al*. 2001 as *Sphingobium xenophaga* comb. nov. and *Sphingopyxis taejonensis* comb. nov., respectively. *Int J Syst Evol Microbiol* **56**, 667-670.

Smith-Grenier, L. L. & Adkins, A. (1996). Isolation and characterization of soil microorganisms capable of utilizing the herbicide diclofop-methyl as a sole source of carbon

301 and energy. *Can J Microbiol* **42**, 221-226.

302

303 **Stolz, A., Schmidt-Maag, C., Denner, E. B. M., Busse, H.-J., Egli, T. & Kämpfer P.**
304 **(2000)**. Description of *Sphingomonas xenophaga* sp. nov. for strains BN6^T and N,N which
305 degrade xenobiotic aromatic compounds. *Int J Syst Evol Microbiol* **50**, 35-41.

306

307 **Takeuchi, M., Hamana, K., & Hiraishi, A. (2001)**. Proposal of the genus *Sphingomonas*
308 *sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the
309 basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405-
310 1417.

311

312 **Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).**
313 The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment
314 aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876-4882.

315

316 **Tindall, B. J. (1990).** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol*
317 *Lett* **66**, 199-202.

318

319 **Ushiba, Y., Takahara, Y. & Ohta, H. (2003)** *Sphingobium amiense* sp. nov., a novel
320 nonylphenol-degrading bacterium isolated from a river sediment. *Int J Syst Evol Microbiol*
321 **53**, 2045-2048.

322

323 **Wilson, K. (1994).** Preparation of genomic DNA from bacteria, In. Current protocols in
324 molecular biology. pp. 241 - 245. Edited by F.M. Ausubel, R. Brent, R.E. Kingston, D.D.
325 Moore, J.G. Seidman, J.A. Smith & K. Struhl. New York: John Wiley & Sons Inc.

Wittich, R.-M., Strömpl, C., Moore, E. R. B., Blasco, R. & Timmis, K. N. (1999). Interaction of *Sphingomonas* and *Pseudomonas* strains in the degradation of chlorinated dibenzofurans. *J Ind Microbiol Biotechnol* **23**, 353-358.

Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99-119.

Yabuuchi, E., Yamamoto, H., Terakubo, S., Okamura, N., Naka, T., Fujiwara, N., Kobayashi, K., Kosako, Y. & Hiraishi, A. (2001) Proposal of *Sphingomonas wittichii* sp. nov. for strain RW1^{T}, known as a dibenzo-*p*-dioxin metabolizer. *Int J Syst Evol Microbiol* **51**, 281-292.

Figure legends:

Fig..1. Unrooted neighbour-joining dendrogram of the phylogenetic relationships between *Sphingobium aromaticiconvertens* sp. nov. RW16^T, eight *Sphingobium* species, *Sphingomonas cloacae,* and type species of the genera *Sphingomonas, Sphingopyxis, Novosphingobium and Sphingosinicella* based on a distance matrix analysis of the 16S rDNA sequences. Accession numbers are given in parentheses. Bootstrap percentages are indicated at tree brancing points and the scale bar presents substitutions per nucleotide.

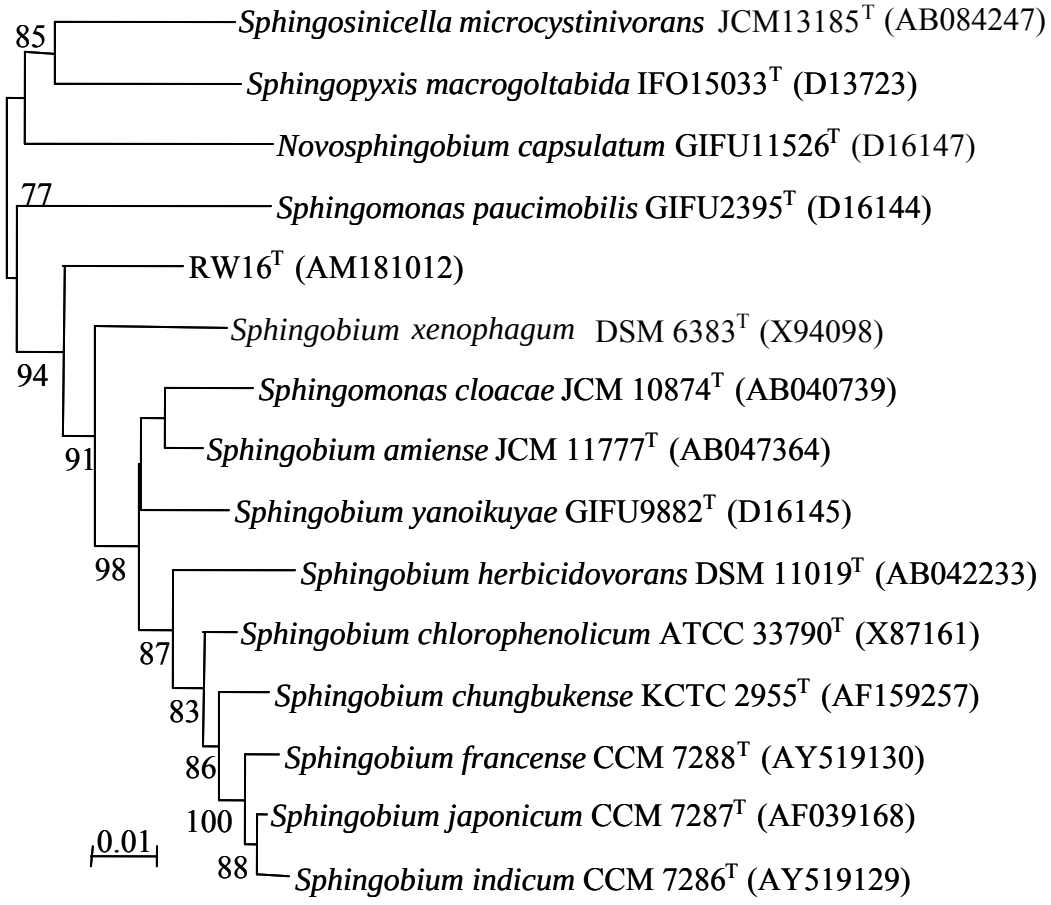

Table 1. Differentiating biochemical characteristics of strain RW16^T, eight *Sphingobium* species, *Sphingomonas cloacae* and *Sphingomonas paucimobilis*

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$^{T;}$ 12, *Sphingomonas paucimobilis XXXX*. Data for strains 2-6 are from Ushiba *et al*. (2003), for strains 6 and 7 from Kim *et al*. (2000), for strains 8 -10 from Pal *et al*. (2005), for strain 11 from Stolz *et al*.(2000) Symbols: + = positive reaction, - = negative reaction, ND = not determined

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 → nitrite	-	-	-	-	-	-	-	ND	ND	ND	ND
ß-galactosidase	+	-	-	+	-	-	-	ND	ND	ND	-

364 **Table 2**: Cellular fatty acids of *Sphingobium* species and *Sphingomonas cloacae*. The data for *S. amiense* (Ushiba *et al*., 2003), *S. chungbukense*

365 (Kim *et al*., 2000), *S. cloacae* (Fujii *et al*., 2001), *S. francense*, *S. indicum*, and *S. japonicum* (Pal *et al*., 2005), were taken from the literature for

366 comparison.

Species	14:0	16:0	17:0	18:0	16:1 ω5c	16:1 ω9c	17:1 ω6c	17:0 cyc7,8	18:1 ω5c	12:0 2OH	13:0 2OH	14:0 2OH	16:0 2OH	SF 4[a]	SF 7
Sphingobium amiense JCM 11777^T		8					2					7		13	62
Sphingobium chlorophenolicum ATCC 33790^T	0.5	9.5			2.4		6.4		2.5			9.4		9.6	60.1
Sphingobium chungbukense DJ77^T		12			1		2		3			9		6	67
Sphingomonas cloacae JCM 10874^T	0.1	7.2	0.3	0.7	3.3	0.7			1.3			2.8	0.2		80
Sphingobium francese CCM 7288^T	0.2	16.5		0.4	1.2				1.1			5.1	0.2		62.5
Sphingobium herbicidivorans DSM 11019^T		7.4			1.6		1.7		2.0	1.0	2.6	5.5		13.4	68.3
Sphingobium indicum CCM 7286^T		19.0		0.9	0.9				1.6			6.8	0.2	0.2	55.7
Sphingobium japonicum CCM 7287^T	0.2	10.0			2.2				1.4			4.3			66.7
RW16^T	1.2	7.8						2.6	1.6			11.8			74.8
Sphingobium xenophagum DSM 6383^T		8.0			2.0		2.5		1.6			6.7		23.9	55.2
Sphingobium yanoikuyae IFO 15102^T		11.2			2.7		3.2		2.1			7.1	0.9	16.3	56.0

367

368 [a]Fatty acid nomenclature: The "summed feature" SF 4 consists of one or more of the following fatty acids: 16:1 ω7c and 15:0 iso 2OH. SF 7

369 consists of one or more of the following isomers: 18:1 ω7c, 18:1 ω9t, and /or 18:1 ω12t.

370 **Table 3.** a) Distribution of polar lipids in type species and the new species of the genus *Sphingobium*.

371 Determined by TLC

Species	PME	PE	PG	DPG	PDE	PC	SGL	APL1	PL1	PL2	PL3	PL4	GL1	GL2	GL3	GL4	L1	L2
S. chlorophenolica ATCC 33790^T	++	++	++	++	++	+	++	-	-	+	+	-	-	-	-	-	-	-
S. herbicidivorans DSM 11019^T	+	++	++	++	++	++	++	-	-	-	+	-	-	-	-	-		
RW16^T	+	+	+	++	+	-	++	+	+	+	+	-	++	+	-	-	-	-
S. xenophagum BN6^T	+	++	++	++	++	+	++	-	-	-	+	++	-	-	-	-	+	-
S. yanoikuyae IFO 15102^T	+	++	++	++	++	+	++	+	+	-	-	-	++	-	-	-	-	-

372

373 b) FAB-(-) of the phospholipids. Lipids identified by CID-MS are in bold.

Mass	**686**	**688**	**700**	**714**	**729**	**741**	**743**	**745**	**757**	**771**	**774**	**785**	**788**	**799**	**802**	**1056**	**1072**	**1268**	**1296**	**1388**
Phospholipid	**PA**	**PA**	**PA**	**PA**	**PME**	**PDE**	**PDE**	**PDE**	**PC**	**PDE**	**PG**	**PC**	**PG**	**PC**	**PG**					
Fatty acids	19:1	19:1	18:1	19:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	18:1	19:1	19:1	19:1					
	16:1	16:0	18:1	18:1	16:1	16:2	16:1	16:0	16:1	18:1	18:1	18:1	18:1	18:1	19:1					
RW16^T	-	-	-	-	-	-	X	-	X	X	X	X	X	X	X	X	X	X	X	X
S. yanoikuyae JCM 7371^T	-	-	X	-	X	-	X	X	X	X	-	X	-	-	-	-	-	-	-	-

374 PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol, PDE,
375 phosphatidyldimethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; APL1, unidentified aminophospholipid; PL1, PL2, PL3, unidentified phospholipids;
376 GL1, GL2, GL3, GL4, unidentified glycolipids; PGL1, unidentified phosphoglycolipid; L1, L2, unidentified lipids. ++, present in major amounts; +, present in minor
377 amounts; -, not detected. Table 3b: 1st line = molecular mass of the lipid; 2nd line: PA = phosphatidyl acid (the two fatty acids attached to the respective phospholipid are
378 listed in lines 3-4).

Supplementary figure A. Phylogenetic relationships calculated between RW16^T, *Sphingomonas cloacae*, *Sphingobium* species and *Sphingomonas, Sphingopyxis, Novosphingobium* and *Sphingosinicella* type species using UPGMA with Kimura-2 parameter model. Bootstrap values are indicated at tree branching points and the scale bar presents percentage of substitutions per nucleotide.