Proposal of *Sphingomonas fennica* sp. nov. and *Sphingomonas haloaromaticamans* sp. nov., outliers of the genus *Sphingomonas*

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

[1]Bereich Mikrobiologie, Helmholtz Zentrum für Infektionsforschung (formerly GBF), 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, F-40500 Jyväskylä, Finland,

[55]Línea de Degradación de Tóxicos Orgánicos, Depto. de Protección Ambiental, Estación Experimental del Zaidín – EEZ, Consejo Superior de Investigaciones Científicas, Calle Profesor Albareda 1, E - 18008 Granada, Spain

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* Author for correspondence:

Dr. Wolf-Rainer Abraham

Helmholtz Center for Infection Research (formerly GBF)

Chemical Microbiology

Inhoffenstrasse 7

38124 Braunschweig

Germany

Phone: *49-531-6181 4300

Fax: *49-531-6181 4699

E-mail: wab@gbf.de

Summary:

Bacterial isolates obtained from polychlorophenol-contaminated sites in Finland (strain K101^T) and from a Dutch drinking water well (strain A175^T), were taxonomically characterized. 16S rRNA gene sequence analysis, determination of DNA G+C content, physiological characterisation, estimation of the ubiquinone and polar lipid patterns, and fatty acid composition revealed that strains K101^T and A175^T were similar to *Sphingomonas wittichii* RW1^T but showed also pronounced differences. The DNA G+C content of the two strains were 63.6 and 66.1 mol %, repectively. On the basis of these results, two novel *Sphingomonas* species are descibed, for which we propose the names *Sphingomonas haloaromaticamans* sp. nov. with the type strain A175^T (DSM 13477^T, CCUG 53463^T) and *Sphingomonas fennica* sp. nov. with the type strain K101^T (DSM 13665^T, CCUG 53462^T).

From different habitats several bacterial strains have been isolated which grow at the expense of chlorinated aromatic compounds, many of them considered as xenobiotics. Studies have been conducted to elucidate the environmental behaviour of these isolates (White *et al*., 1996), many of them originally were assigned to the genus *Sphingomonas* (Balkwill *et al*., 1997; Karlson *et al*., 1995; Moore *et al*., 1993; Nohynek *et al*., 1995, 1996; Stolz *et al*., 2000; Zipper *et. al*., 1996) but, with the exception of *Sphingomonas wittichii* all of them were reclassified as species of the genera *Sphingobium* or *Novosphingobium* (Takeuchi *et al*., 2001; Pal *et al*., 2006). There are some indications that the intermediary halocatechols, especially 4-fluoro- and 4-chlorocatechol, originating from the bacterial breakdown of some haloaromatics, can be mineralized through the proposed novel pathway involving protoanemonin (Blasco *et al*., 1995; Nikodem *et al*., 2003; Wittich *et al*., 1999). This is possible in several strains expressing only genes encoding the ortho-pathway for the breakdown of catechol. Inducing extradiolically cleaving catechol dioxygenase activity would lead to the misrouting of halogenated intermediates in those strains lacking a functional chlorocatechol pathway. In sphingomonads, a chlorocatechol pathway (in sensu strictu) was detected only in the 1,4-dichlorobenzene-mineralizing strain A175^T and may be found in some chlorophenoxy herbicides degrading isolates (Ka *et al*., 1994), and in Gram-positive bacteria (Konig *et al*., 2004).

Strain A175^T was isolated from a mixture of various soil and water samples with 1,4-dichlorobenzene as sole carbon and energy source (Schraa et al., 1986). The strain was initially identified as *Alcaligenes* sp. and only later it was discovered that the strain belongs to the *Sphingomonadaceae* (Kosako *et al*., 2000). Strain K101^T was isolated from 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol contaminated boreal groundwater in Finland and was able to degrade all those compounds (Männistö *et al*., 1999). Here, we report results of a polyphasic study on these isolates which currently have been assigned to the genus

73 *Sphingomonas* (Yabuuchi *et al*., 1990; Takeuchi *et al.*, 2001).

74 Biomass for extraction of quinones, polar lipids and polyamines were grown on PYE
75 medium (Busse *et al*., 2005) and for extraction of fatty acids it was grown on trypticase soy agar.
76 Liquid and solid LB or R2A medium was used for the purpose of culturing strains for other
77 subsequent taxonomic tests in this study. Solid mineral salts medium plates were used to check
78 the degradation of individual organic carbon sources at a concentration of organic carbon
79 corresponding to about 25 mM single carbon atom. Cultures were grown aerobically at 28°C.
80 Cell morphology and dimensions were determined by phase-contrast microscopy. Cells were rod-
81 shaped, 0.8-1.5 µm in length and 0.4-0.6 µm in diameter. They showed a tendency to grow in
82 rosette-like formation. However, when grown on selective media most organisms tend to grow as
83 branched thread-like/hyphae-like aggregates.

84 Procedures for physiological and biochemical characterizations were those described
85 earlier (Kämpfer & Altweg, 1992; Kämpfer *et al*., 1991). The results using API galleries
86 (bioMérieux) and Biolog substrate utilization tests are given in detail in the species description.
87 Furthermore, strain A175^T has been reported to grow on 1,4-dichlorobenzene but not on chloro-
88 phenols (Schraa *et al*., 1986) and strain K101^T was found to use tri-, tetra- and pentaphenols
89 (Männistö *et al*., 1999).

90 Interestingly, strain K101^T is capable of assimilating only two of the long list of carbon
91 sources tested, namely acetate and DL-3-hydroxybutyrate, and is thus rather unique in this genus
92 (Kämpfer *et al*., 1997; Denner *et al*., 1999). In this high specialization it resembles the strains of
93 another sphingomonad species *Novosphingobium lentum* isolated from a chlorophenol-degrading
94 bioreactor purifying the water of the same aquifer (Tiirola *et al* ., 2005).

95 For isolation of genomic DNA for G+C content determination, bacterial DNA was
96 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. With 66.1 % for $A175^T$ and 63.6 % for $K101^T$ the values are within the range of the genera of the *Sphingomonadaceae* but do not allow an affiliation to one of the genera.

For the amplification of the 16S rRNA gene by polymerase chain reaction single colonies were boiled in 100 µl of TE buffer for about 10 min at 95°C to obtain DNA. 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 16S rRNA gene sequences of the strains $A175^T$ and $K101^T$ have been previously deposited at the EMBL database under the accession numbers X94101 (Nohynek *et al*., 1996) and AJ009706 (Männistö et al., 1999), respectively. The sequences were aligned using ClustalX software (Thompson *et al*. 1997). Tree topologies were reconstructed with Neighbour-Joining algorithm with 1000 bootstrap replications, according to Junca and Pieper (2004) (Fig. 1), and the UPMGA algorithm with Kimura-2 parameter was calculated with the software MEGA 3.1. (Kumar *et al.*, 2004) (Supplement Fig. 1) using the EMBL database (Kanz *et al*., 2005). The 16S rRNA gene sequence of strain $A175^T$ showed 93.3 % similarity to the 16S rRNA gene sequence of *Sphingomonas paucimobilis* GIFU 2395^T, 96.0 % to strain $K101^T$ and 96.7 % to the closest established species, *S. wittichii* $RW1^T$ (Yabuuchi *et al*., 2001). These relatively low sequence similarities indicate that strain $A175^T$ is a novel species. Strain $K101^T$ possessed a 16S

121 rRNA gene sequence similarity of 93.1 % to *S. paucimobilis* GIFU 2395^T and 95.8 % to *S.*
122 *wittichii* RW1^T.

123 The procedures used for extraction and HPLC analysis of bacterial polyamines were
124 performed as described by Busse and Auling (1988) and Busse *et al*. (1997). Strains A175^T
125 (Busse *et al*., 1999) and K101^T represent species containing *sym*-homospermidine as the major
126 polyamine and they are sharing this characteristic with the other species of *Sphingomonas* sensu
127 stricto (Busse *et al.,* 1999; Takeuchi *et al.,* 2001) and *Sphingosinicella* (Geueke *et al.*, in press).
128 With respect to its unusually high content of cadaverine strain A175^T appeared to be unique in
129 the entire genus (Suppl. Table B). Respiratory quinones were extracted as reported by Ventosa *et*
130 *al*. (1993) and Altenburger *et al*. (1996). While strain A175^T formed only ubiquinone Q-10, strain
131 K101^T contained additionally small amounts of Q-9 (2%).

132 Gas chromatography was used to analyse the fatty acid profiles of the strains, as described
133 previously (Kämpfer et al., 1992). $C_{16:1\omega9c}$ is shared by *S. wittichii* RW1^T and K101^T only, as well
134 as $C_{16:1\omega11c}$, which also has not been found within the genus *Sphingomonas* yet (Busse *et al*.,
135 1999). $C_{18:0}$ was found in *S. wittichii* RW1^T, A175^T and K101^T; the latter is lacking $C_{16:1\omega5c}$,
136 $C_{17:1\omega6c}$, $C_{17:1\omega8c}$, $C_{18:1\omega5c}$, and those fatty acids in summed feature 4 which are present in strain
137 A175^T (Table 2).

138 Polar lipids were extracted according to Tindall (1990) and analysed by two-dimensional
139 thin layer chromatography. Unique polar lipid profiles distinguished A175^T and K101^T from each
140 other and from *S. wittichii* RW1^T. Lack of phosphatidylmonomethylethanolamine and
141 phosphatidyldimethylethanolamine in the extract of A175^T differentiated it from *S. wittichii*
142 RW1^T (Busse et al., 1999) and strain K101^T. Lack of detectable amounts of phosphatidylcholine
143 and the unknown lipids PL3, GL1 and GL4 in the extract of K101^T was unique among these
144 closely related strains (Suppl. Table A.a).

Lipids were extracted by a modified Bligh-Dyer method (Fredrickson et al., 1986), followed by analysis in the mass spectrometer using fast atom bombardment (FAB-MS) ionisation recorded in the negative mode of selected fractions as already described by Abraham *et al*. (1997). The analysis of the individual phospholipids by mass spectrometry revealed that the $C_{18:1}$-$C_{16:1}$-phosphatidyl *N,N*-dimethyl-ethylamine (743 Da), the corresponding -cholin (756 Da), $C_{18:1}$-$C_{18:1}$-phosphatidic acid (700 Da), the corresponding -*N,N*-dimethyl-ethylamine (771 Da) and -choline (785 Da) are formed by nearly all *Sphingomonadaceae* investigated. Interestingly, for the separated group of strains in the *Sphingomonas* cluster, *S. wittichii* RW1^T and strains K101^T and A175^T, some lipids could be identified occurring preferently in these strains. These are $C_{19:1}$-$C_{16:0}$-phosphatidic acid (688 Da) and -glycerol (762 Da), $C_{18:1}$-$C_{16:1}$-*N*-methyl-ethylamine (729 Da) and $C_{18:1}$-$C_{16:2}$-phosphatidyl-*N,N*-dimethylethyl-amine (741 Da) (Suppl. Table A.b).

Conclusions

On the basis of the 16S rRNA sequences, strains A175^T and K101^T are only remotely related to the other *Sphingomonas* species. Although strain A175^T is more closely related to *S. wittichii* RW1^T than to K101^T on the basis of 16S rRNA gene sequence comparison, strain A175^T differs significantly from these sphingomonads in its polyamine pattern (Suppl. Table B).

Our data do not support the notion of Takeuchi et al. (2001) that members of the genera *Sphingomonas* and those of *Sphingobium*, *Novosphingobium* and *Sphingopyxis* can be discerned by the presence of sym-homospermidine and the absence of spermidine in *Sphingomonas* species while species of the other three genera possess spermidine but not homospermidine (Suppl. Table B). Both polyamines were detected in *Sphingomonas wittichii* RW1^T, and strains A175^T, K101^T but also in the type strains of *Sphingomonas asaccharolytica*, *Sphingomonas paucimobilis,*

169 *Sphingomonas trueperi, Sphingomonas abaci*, *Sphingomonas panni*, *Sphingomonas echinoides*
170 and *Sphingomonas pituitosa* (Busse *et al*., 1999, 2005; Denner *et al*., 1999, 2001). However, all
171 species of *Sphingomonas senu stricto* are characterized by the predominant polyamine *sym*-
172 homospermidine. These results show that species of *Sphingomonas* have mainly *sym*-
173 homospermidine and some of them produce minor quantities of spermidine as well. Species of
174 *Sphingobium*, *Novosphingobium* and *Sphingopyxis* produce no *sym*-homospermidine. However,
175 *sym*-homospermidine also characterizes species of the genus *Sphingosinicella* (Maruyama *et al*.,
176 2006; Geueke *et al*., in press). Although strains K101^T and A175^T share most signature
177 nucleotides proposed by Takeuchi *et al*. (2001) for differentiation of *Sphingomonas* senu stricto
178 from related genera, they are also found in the species of *Sphingosinicella* (Maruyama *et al*.,
179 2006; Geueke *et al*., in press). In conclusion neither the polyamine patterns nor the signature
180 nucleotides allow assignment of K101^T and A175^T to any of the two genera. The phylogenetic
181 distance to representatives of *Sphingosinicella* and close relatedness to *Sphingomonas wittichii* as
182 well as differences in the signatures identified for *Sphingosinicella* by Geueke *et al*. (in press)
183 support assignment of K101^T and A175^T to the genus *Sphingomonas.* Therefore, we propose
184 *Sphingomonas fennica* sp. nov. K101^T and *Sphingomonas haloaromaticamans* sp. nov. A175^T.
185
186 **Description of *Sphingomonas haloaromaticamans* sp. nov.**
187 The description of *Sphingomonas haloaromaticamans* (ha.lo.a.ro.ma.tic.a'mans. N.L. n.
188 haloaromaticum, haloaromatic, class of chemical compound; L. part. adj. amans, loving; N.L.
189 part. adj. haloaromaticamans, loving haloaromatics). *Sphingomonas haloaromaticamans*
190 (halo.aro.ma.ti.ci'amans. Chem. n. haloaromatic, class of chemical compound; L. adj. *amans*,
191 loving; *haloaromaticamans*, loving haloaromatics) is the same as that given for the genus with
192 the following additional characteristics.

Cells are rod-shaped, 0.8 - 1.5 :m in length and 0.4 - 0.6 :m in diameter. Colonies yellow colored, cells rod-shaped. N-Acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-galactose, D-glucose, D-maltose, alpha-D-melibiose, D-xylose, acetate, propionate, azelate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-alanine, L-aspartate, L-histidine, L-leucine, L-proline, and L-tryptophane are used as substrate, but not D-fructose, gluconate, D-mannose, L-rhamnose, sucrose, salicin, D-trehalose, maltitol, D-mannitol, D-sorbitol, *cis*-aconitate, adipate, 4-aminobutyrate, citrate, fumavate, L-malate, L-ornithine, L-phenylalanine, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. Esculin, pNP-α-D-glucopyranoside, pNP-ß-D-glucopyranoside, bis-pNP-phosphate, pNP-phenyl phosphonate, 2-deoxythymidine-5'-pNP-phosphate, L-alanine-pNA, L-glutamate-γ-3-carboxy-pNA, and L-proline-pNA are hydrolysed but pNP-ß-D-galactopyranoside, pNP-ß-D-glucuronide, pNP-phosphoryl choline not. Nitrate is not reduced. Main polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and sphingoglycolipid, while phosphatidylcholine, the unidentified phospholipid PL3 and the unidentified glycolipid GL4 are minor constituents. The species is characterized by the major fatty acids $C_{18:1}$ (Sum7) and $C_{16:1}$ (Sum7), minor fatty acids are $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{18:0}$, $C_{16:1}\omega5c$, $C_{17:1}\omega6c$, $C_{17:1}\omega8c$, and $C_{18:1}\omega5c$; the major hydroxy-fatty acid is 2-hydroxy-$C_{14:0}$ together with minor amounts of 2-hydroxy-$C_{15:0}$. Major polyamine is homospermidine, minor polyamines diaminopropane, putrescine, cadaverine, spermidine and spermine. The species produces only ubiquinone Q-10. The G+C content is 66.1 %. The species can grow between 10°-40°C with optimal growth between 30°-37°C, optimal pH range is 5-8. Isolated from water and soil samples, the Netherlands, as degrader of benzene, catechol, chlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene. The type strain is $A175^T$ (= DSM 13477^T = CCUG 53463^T).

Description of *Sphingomonas fennica* sp. nov.

The description of *Sphingomonas fennica* (fen.ni'ca. N.L. fem. adj. fennica, pertaining to Finland) is the same as that given for the genus with the following additional characteristics.

Colonies colored light yellow but the colour is not stable during subcultivation on rich medium, motile and short plump, rod-shaped cells 0.5 - 0.9 μm by 0.9 - 1.5 μm. The species reduces nitrate to nitrogen. Acetate and DL-3-hydroxybutyrate are used as substrate but not N-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-mannose, D-maltose, α-D-melibiose, L-rhamnose, sucrose, salicin, D-trehalose, D-xylose, maltitol, D-mannitol, D-sorbitol, propionate, *cis*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, fumavate, DL-lactate, L-malate, pyruvate, L-alanine, L-aspartate, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-tryptophane, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. pNP-phosphoryl choline, 2-deoxythymidine-5'-pNP-phosphate, and L-alanine-pNA are hydrolysed but esculin, pNP-ß-D-galactopyranoside, pNP-ß-D-glucuronide, pNP-a-D-glucopyranoside, pNP-ß-D-glucopyranoside, bis-pNP-phosphate, pNP-phenyl phosphonate, L-glutamate-γ-3-carboxy-pNA and L-proline-pNA not. Polar lipids are phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidyldimethylethanolamine and sphingoglycolipid. The species is characterized by the major fatty acids $C_{18:1}$ (Sum7), $C_{16:1\omega9c}$ and $C_{16:0}$, minor fatty acids are $C_{14:0}$, $C_{18:0}$, and $C_{16:1\omega11c}$; the major hydroxy-fatty acid is 2-hydroxy-$C_{14:0}$ with minor amounts of 2-hydroxy-$C_{15:0}$ and 2-hydroxy-$C_{16:0}$. Major polyamine is homospermidine, minor polyamines are putrescine, cadaverine, spermidine and spermine. The species produces ubiquinone Q-10, but small amounts (2%) of Q-9 can also be detected, the G+C content is 63.6 %. The species can grow between 10°-37°C with optimal growth between 20°-30°C, pH range is 5-8, optimal pH 7-8. Isolated from polychlorophenol-contaminated groundwater adjacent to a sawmill, Southern

241 Finland, as degrader of 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol.
242 The environment of original isolation was cold (7-8°C), oxygen-deficient, humic (4-23 mg DOC
243 l^{-1}) and slightly acidic (pH 6-6.5). The type strain is $K101^T$ (= DSM 13665^T = CCUG 53462^T).

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Figure legend:

Figure1. Unrooted neighbour-joining dendrogram of the phylogenetic relationships between *Sphingomonas haloaromaticamans* sp. nov. A175^{T}, *Sphingomonas fennica* sp. nov. K101^{T}, all valid *Sphingomonas* species, and type species of the genera *Sphingobium, 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

Supplementary Figure A. Dendrogram of the phylogenetic relationships between *Sphingomonas haloaromaticamans* sp. nov. A175^{T}, *Sphingomonas fennica* sp. nov. K101^{T}, all valid *Sphingomonas* species, and type species of the genera *Sphingobium, Sphingopyxis, Novosphingobium and Sphingosinicella* 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.based on a distance matrix analysis of the 16S rDNA sequences. Accession numbers are given in parentheses.

457 Figure 1

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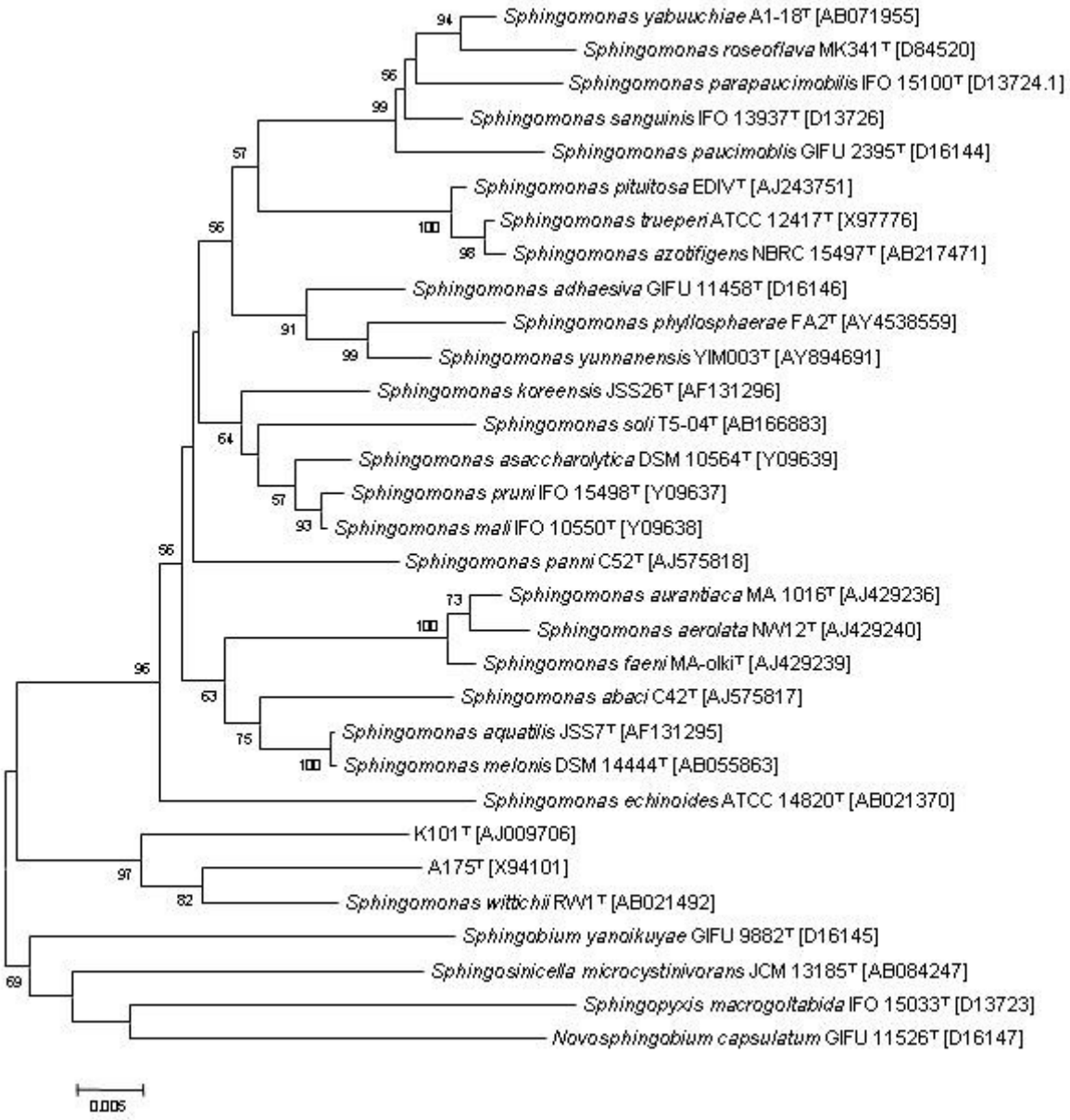

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462 **Supplementary Figure A**.

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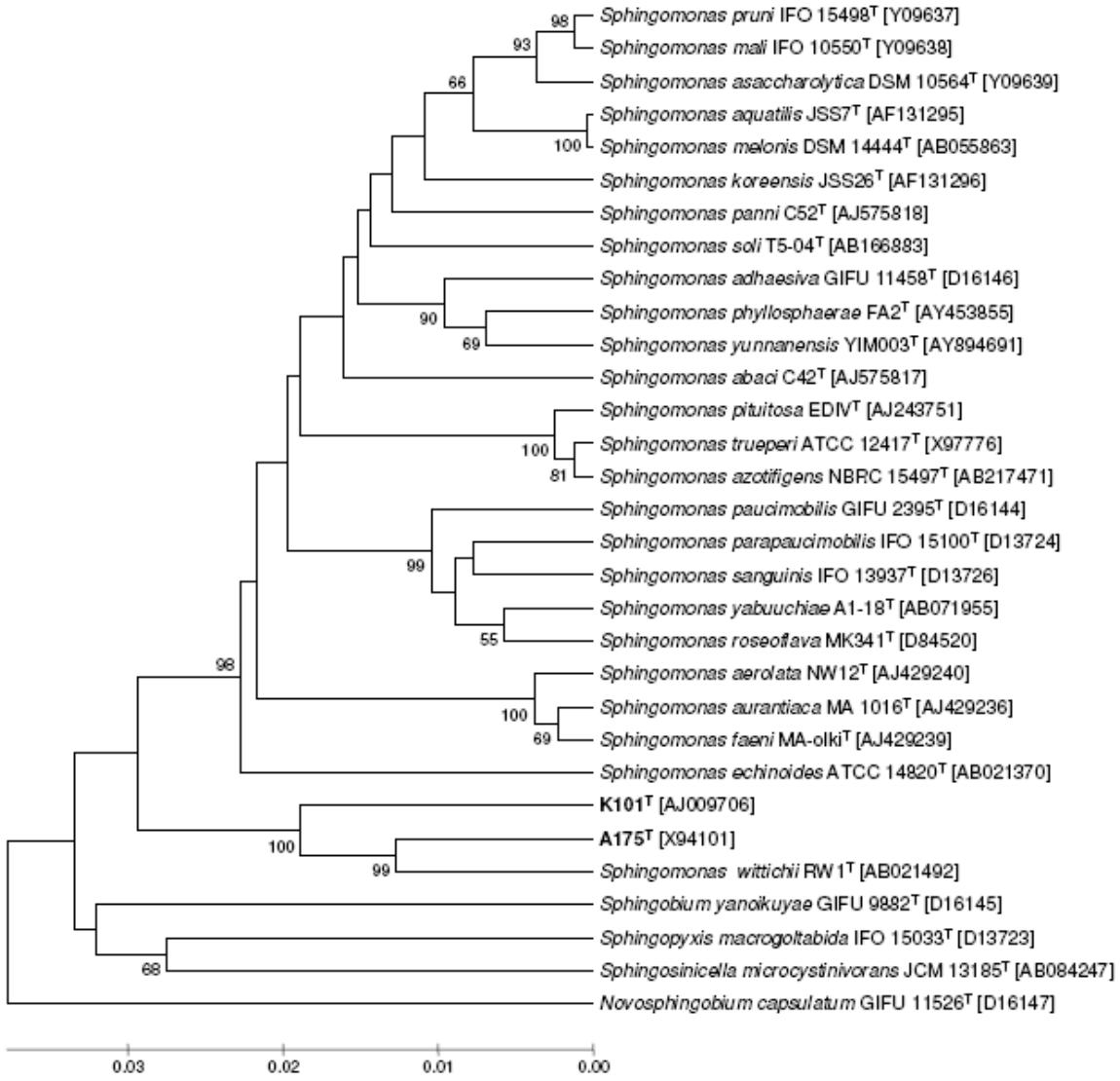

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Table 1. Differentiating biochemical characteristics of strains A175^T, K101^T and *Sphingomonas wittichii* RW1^T

Strains: 1, A175^T; 2, K101^T; 3, *Sphingomonas wittichii* RW1^T Symbols: + = positive reaction, - = negative reaction.

Test	**1**	**2**	**3**
Assimilation of:			
N-Acetyl-D-glucosamine, L-arabinose, *p*-arbutin, azelate, D-cellobiose, D-galactose, D-glucose, D-maltose, ∀-D-melibiose, D-xylose	+	-	-
Phenylacetate, L-phenylalanine, salicin	-	-	+
L-Alanine, L-aspartate, L-histidine, L-leucine, L-tryptophane, propinate	+	-	+
Adipate, 4-aminobutyrate, fumarate L-malate, L-ornithine	-	-	-
DL-Lactate, pyruvate	+	-	+
L-Proline	+	-	-
Hydrolysis of :			
Esculin, pNP-a-D-glucopyranoside, pNP-ß-D-glucopyranoside, pNP-phenyl phosphonate, L-proline-pNA,	+	-	-
pNP-ß-D-glucuronide	-	-	+
Bis-pNP-phosphate, L-glutamate-(-3-carboxy-pNA	+	-	+
pNP-phosphoryl choline	-	+	-

Table 2: Cellular fatty acids of *Sphingomonas* species.

Species	14:0	15:0	16:0	18:0	16:1 ω5c	16:1 ω9c	16:1 ω11c	17:1 ω6c	17:1 ω8c	18:1 ω5c	14:0 2OH	15:0 2OH	16:0 2OH	Summed feature 4	Summed feature 7
S. wittichii RW1^T	1.7		12.8	0.3	6.0	11.0	3.3	0.7		1.4	7.4	<0.1	<0.1	9.3	55.6
S. sp. A175^T	0.7	0.8	9.2	0.7	5.2			7.1	0.9	1.6	8.3	0.6		11.0	51.6
S. sp. K101^T	0.5		11.4	0.2		18.5	2.1				8.6	0.4	1.0		54.3

The "summed feature" represents groups of two or three fatty acids that cannot be separated by gas-liquid chromatography with the MIDI system. Summed feature 4 consists of one or more of the following fatty acids: 16:1 ω7c and 15:0 iso 2OH. Summed feature 7 consists of one or more of the following isomers: 18:1 ω7c, 18:1 ω9t, and /or 18:1 ω12t (*cis* and *trans* isomers are indicated by the suffixes c and t, respectively).

Supplementary Table A. a) Distribution of polar lipids in type strains and the new species of the genus *Sphingomonas* determined by TLC.

Species	PME	PE	PG	DPG	PDE	PC	SGL	APL1	PL1	PL2	PL3	PL4	GL1	GL2	GL3	GL4	L1	L2
S. paucimobilis DSM 1098^T	-	++	++	+	-	++	++	-	-	-	+	-	-	+	-	-	-	-
S. parapaucimobilis IFO 15100^T	+	++	++	++	+	++	+	-	-	+	+	-	++	+	-	-	-	-
S. wittichii RW1^T	++	++	++	++	++	+	++	-	-	-	+	-	+	-	-	+	-	-
***S.* sp. A175^T**	-	++	++	++	-	+	++	-	-	-	+	-	+	-	-	+	-	-
***S.* sp. K101^T**	+	+	+	+	+	-	+											

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

m/z	**671**	**673**	**685**	**687**	**699**	**713**	**728**	**740**	**742**	**744**	**747**	**756**	**758**	**759**	**761**	**770**	**773**	**784**	**787**	**798**	**801**	**830**	**917**	**931**
Phospholipid	**?**	**PA**	**PA**	**PA**	**PA**	**PA**	**PME**	**PDE**	**PDE**	**PDE**	**PG**	**PC**	**?**	**PG**	**PG**	**PDE**	**PG**	**PC**	**PG**	**PC**	**PG**	**?**	**?**	**?**
Fatty acids		16:0	19:1	19:1	18:1	19:1	18:1	18:1	18:1	18:1	18:1	18:1		19:1	19:1	18:1	18:1	18:1	19:1	19:1	19:1			
		18:1	16:1	16:0	18:1	18:1	16:1	16:2	16:1	16:0	16:0	16:1		16:1	16:0	18:1	18:1	18:1	18:1	18:1	19:1			
S. paucimobilis DSM 1098^T	x	**X**	-	-	**X**	x	-	-	x	**X**	x	x	x	-	-	**X**	**X**	x	x	-	-	x	-	x
S. parapaucimobilis JCM 7510^T	x	**X**	x	-	**X**	-	-	-	x	x	x	x	x	-	-	x	**X**	**X**	x	-	-	x	-	x
S. wittichii RW1^T	x	x	x	x	**X**	x	x	x	**X**	**X**	**X**	x	-	x	x	**X**	**X**	**X**	x	x	x	-	-	-
***S.* sp. K101^T**	x	-	-	-	x	-	**X**	**X**	**X**	-	x	**X**	x	-	-	**X**	**X**	x	x	-	-	-	-	-
***S.* sp. A175^T**	x	-	**X**	**X**	**X**	**X**	-	x	**X**	-	-	**X**	-	**X**	**X**	**X**	**X**	x	**X**	**X**	x	x	-	x

PA = phosphatidyl acid PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol, PDE,

phosphatidyldimethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; APL1, unidentified aminophospholipid; PL1, PL2, PL3, unidentified phospholipids; GL1, GL2, GL3, GL4, unidentified glycolipids; PGL1, unidentified phosphoglycolipid; L1, L2, unidentified lipids. ++, present in major amounts; +, present in minor amounts; -, not detected.

Supplementary Table B: Polyamine pattern of *Sphingomonas* species. The concentration of individual polyamine is given as :moles x g^{-1} (dry weight). Abbreviations: DAP = diaminopropane; PUT = putrescine; CAD = cadaverine; SPD = spermidine; HSPD = homospermidine; SPM = spermine.

Species	DAP	PUT	CAD	SPD	HSPD	SPM
S. wittichii RW1^T		0.3		2.2	48.9	1.3
S. haloaromaticamans A175^T	1.00	1.0	7.1	6.6	50.1	1.9
S. fennica K101^T		<0.1	<0.1	0.2	39.9	<0.1