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

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Bacterial isolates obtained from polychlorophenol-contaminated sites in Finland (strain K101T) and from a Dutch drinking water well (strain A175T) were characterized taxonomically. 16S rRNA gene sequence analysis, determination of DNA G+C content, physiological characterization, estimation of the ubiquinone and polar lipid patterns and fatty acid content revealed that strains K101T and A175T were similar to **Sphingomonas wittichii** RW1T but also showed pronounced differences. The DNA G+C contents of the two novel strains were 63.6 and 66.1 mol%, respectively. On the basis of these results, two novel species of the genus **Sphingomonas** are described, for which the names **Sphingomonas haloaromaticamans** sp. nov. [type strain A175T (= DSM 13477T = CCUG 53463T)] and **Sphingomonas fennica** sp. nov. [type strain K101T (= DSM 13665T = CCUG 53462T)] are proposed.

Several bacterial strains have been isolated from different habitats that are able to grow at the expense of chlorinated aromatic compounds, many of which are considered to be xenobiotics. Several studies have been conducted to elucidate the environmental behaviour of these novel isolates (White et al., 1996) and many of them were originally assigned to the genus **Sphingomonas** (Balkwill et al., 1997; Karlson et al., 1996; Moore et al., 1993; Nohynek et al., 1996a, b; Stolz et al., 2000; Zipper et al., 1996). However, with the exception of **Sphingomonas wittichii**, all of these isolates have since been reclassified as species of the genera **Sphingobium** or **Novosphingobium** (Pal et al., 2006; Takeuchi et al., 2001). There are some indications that the intermediary halocatehols, especially 4-fluorocatechol and 4-chlorocatechol, originating from the bacterial breakdown of some haloaromatics, can be mineralized through a proposed novel pathway involving protoanemonin (Blasco et al., 1995; Nikodem et al., 2003; Wittich et al., 1999). This mechanism is possible in several strains that express only the 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 stricto) has been detected only in the 1,4-dichlorobenzene-mineralizing strain A175T and may also be found in some chlorophenoxy herbicide-degrading isolates (Ka et al., 1994) and in Gram-positive bacteria (Konig et al., 2004).

Strain A175T was isolated from a mixture of various soil and water samples that used 1,4-dichlorobenzene as the sole carbon and energy source (Schraa et al., 1986). The

**Abbreviations:** pNA, p-nitroanilide; pNP, p-nitrophenyl.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains A175T and K101T are X94101 and AJ009706, respectively.

An additional phylogenetic tree and tables detailing polar lipid distribution, FAB of phospholipids and polyamine patterns for the novel species are available with the online version of this paper.
novel strain was initially identified as a member of the genus *Alcaligenes* and only later was it discovered that the strain actually belongs to the family *Sphingomonadaceae* (Kosako et al., 2000). Strain K101T was isolated from 2,4,6-trichlorophenol-, 2,3,4,6-tetrachlorophenol- and pentachlorophenol-contaminated boreal groundwater in Finland and this novel strain was able to degrade all of these compounds (Männistö et al., 1999). Here, we report the results of a polyphasic study of these two novel isolates which are currently assigned to the genus *Sphingomonas* (Takeuchi et al., 2001; Yabuuchi et al., 1990).

Biomass for the extraction of quinones, polar lipids and polyamines was grown on PYE medium (Busse et al., 2005). For the extraction of fatty acids, biomass was grown on trypticase soy agar. Liquid and solid Luria–Bertani (LB) or R2A media were used for culturing the strains for the other taxonomic tests described in this study. Solid mineral salts medium plates were used to check the degradation of individual organic carbon sources at a concentration of organic carbon corresponding to about 25 mM single carbon atom. Cultures were grown aerobically at 28 °C. Cell morphology and dimensions were determined by phase-contrast microscopy. Cells were rod-shaped, 0.8–1.5 μm in length and 0.4–0.6 μm in diameter. Cells of both strains showed a tendency to grow in rosette-like formations. However, when grown on selective media, cells of both novel strains tended to grow as branched thread-like/hyphae-like aggregates.

The procedures used for physiological and biochemical characterizations were those described earlier (Kämpfer & Altwegg, 1992; Kämpfer et al., 1991). The results obtained using API galleries (bioMérieux) and Biolog substrate utilization tests are given in detail in the species description. Strain A175T has been reported to grow on 1,4-dichlorobenzene, but not on chlorophenols (Schraa et al., 1986), and strain K101T has been found to use tri-, tetra- and pentaphenols (Männistö et al., 1999).

Interestingly, strain K101T was capable of assimilating only two of the long list of carbon sources tested, namely acetate and DL-3-hydroxybutyrate, and is thus unique in the genus *Sphingomonas* (Denner et al., 1999; Kämpfer et al., 1997) (Table 1). With this highly specialized characteristic, strain K101T resembles strains of another sphingomonad species, *Novosphingobium lentum*, which was isolated from a chlorophenol-degrading bioreactor purifying water from the same aquifer (Tiirila et al., 2005).

For the isolation of genomic DNA for the determination of G + C content, bacterial DNA was purified using proteinase K lysis, phenol/chloroform extractions and isopropanol precipitation according to Wilson (1994). The purity of the DNA samples was confirmed with caesium chloride gradient centrifugation. DNA G + C content was determined as described by Johnson (1994) using λ phage DNA as a standard. The separation was performed on a Purospher endcapped reverse-phase HPLC column (Merck) of 250 × 4 mm. The mobile phase was 20 mM triethylammonium phosphate in 12 % aqueous methanol at a flow rate of 1 ml min⁻¹ at 22 °C. The DNA G + C contents were 66.1 mol% for strain A175T and 63.6 mol% for strain K101T. These values are within the range commonly found for members of the family *Sphingomonadaceae* but did not allow a clear affiliation to be made to a specific genus.

For the amplification of the 16S rRNA gene by PCR, 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). Reactions were evaluated on an Applied Biosystems 377 Genetic Analyzer and the final contig was assembled using the SEQUENCER program version 4.0.5 (Gene Codes Corporation). The sequences

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
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</thead>
<tbody>
<tr>
<td><strong>Assimilation of:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-d-glucosamine, L-arabinose, p-arbutin, azelate, d-cellobiose, D-galactose, D-glucose, D-maltose, z-D-melibiose, D-xylene</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Phenylacetate, L-phenylalanine, salicin</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>L-Alanine, L-aspartate, L-histidine, L-leucine, L-tryptophan, propionate</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Adipate, 4-aminobutyrate, fumarate, L-malate, L-ornithine</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>D,L-Lactate, pyruvate</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Proline</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin, pNP-α-D-glucopyranoside, pNP-β-D-glucopyranoside, pNP-phenylphosphonate, L-proline-pNA</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>pNP-β-D-glucuronide</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>bis-pNP-phosphate, L-glutamate-γ-3-carboxy-pNA</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>pNP-phosphorylcholine</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>
were matched against the EMBL database (Kanz et al., 2005) using BLAST 2.2.9 (Altschul et al., 1990). The 16S rRNA gene sequences of strains A175<sup>T</sup> and K101<sup>T</sup> have already been deposited in the EMBL database under the accession numbers X94101 (Nohynek et al., 1996a) and AJ009706 (Männistö et al., 1999), respectively. The sequences were aligned using CLUSTAL_X software (Thompson et al., 1997). Tree topologies were reconstructed with the neighbour-joining algorithm with 1000 bootstrap replications, according to the method of Junca and Pieper (2004) (Fig. 1) and the UPGMA algorithm with the Kimura two-parameter model was calculated using MEGA 3.1 software (Kumar et al., 2004) (see Supplementary Fig. S1 in IJSEM Online) using the EMBL database (Kanz et al., 2005). The 16S rRNA gene sequence of strain A175<sup>T</sup> showed 93.3% similarity to that of Sphingomonas paucimobilis GIFU 2395<sup>T</sup>, 96.0% to strain K101<sup>T</sup> and 96.7% to the closest recognized species, S. wittichii RW1<sup>T</sup> (Yabuuchi et al., 2001). These relatively low gene sequence similarities indicate that strain A175<sup>T</sup> represents a novel species. Strain K101<sup>T</sup> showed a 16S rRNA gene sequence similarity of 93.1% to S. paucimobilis GIFU 2395<sup>T</sup> and 95.8% to S. wittichii RW1<sup>T</sup>.

The procedures used for extraction and HPLC analysis of bacterial polyamines were performed as described by Busse & Auling (1988) and Busse et al. (1997). Strains A175<sup>T</sup> (Busse et al., 1999) and K101<sup>T</sup> represent species containing syn-homospermidine as the major polyamine and they share this characteristic with the other species of the genus Sphingomonas sensu stricto (Busse et al., 1999; Takeuchi et al., 2001) and the genus Sphingosinicella (Geueke et al., 2007). Strain A175<sup>T</sup> appeared to be unique in the entire genus with respect to its unusually high content of

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**Fig. 1.** Unrooted neighbour-joining dendrogram of the phylogenetic relationships between Sphingomonas haloaromaticamans sp. nov. A175<sup>T</sup>, Sphingomonas fennica sp. nov. K101<sup>T</sup>, all recognized species of the genus Sphingomonas and the type species of the genera Sphingobium, Sphingopyxis, Novosphingobium and Sphingosinicella based on a distance matrix analysis of the 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap percentages are indicated at tree branching points. Bar, 0.005 substitutions per nucleotide.
cadaverine (see Supplementary Table S2 in IJSEM Online). Respiratory quinones were extracted as reported by Ventosa et al. (1993) and Altenburger et al. (1996). While strain A175\(^T\) formed only ubiquinone Q-10, strain K101\(^T\) also contained small amounts of Q-9 (2%).

GC was used to analyse the fatty acid profiles of the novel strains, as described previously (Kämpfer et al., 1992). The fatty acid \(\text{C}_{16:1}\) (9c) was shared by \textit{S. wittichii} RW1\(^T\) and strain K101\(^T\) only. These two strains also contained fatty acid \(\text{C}_{16:1}\) (10c) and \(\text{C}_{17:0}\) (9c), which has not been found previously within members of the genus \textit{Sphingomonas} (Busse et al., 1999). Fatty acid \(\text{C}_{18:0}\) was found in \textit{S. wittichii} RW1\(^T\), strain A175\(^T\) and strain K101\(^T\). In contrast to strain A175\(^T\), strain K101\(^T\) did not contain fatty acids \(\text{C}_{16:1}\) (9c), \(\text{C}_{17:0}\) (9c), \(\text{C}_{17:1}\) (9c) or \(\text{C}_{18:1}\) (9c) or those fatty acids that comprise summed feature 4 (Table 2).

Polar lipids were extracted according to the method of Tindall (1990) and analysed by two-dimensional TLC. Unique polar lipid profiles distinguished strains A175\(^T\) and K101\(^T\) from each other and from \textit{S. wittichii} RW1\(^T\). Lack of phosphatidylinomonomethylethanolamine and phosphatidyl-dimethylethanolamine in extracts from strain A175\(^T\) enabled this strain to be differentiated from \textit{S. wittichii} RW1\(^T\) (Busse et al., 1999) and strain K101\(^T\). The absence of detectable amounts of phosphatidylcholine and the unknown lipids PL3, GL1 and GL4 in extracts from strain K101\(^T\) was unique among these closely related strains (see Supplementary Table S1a in IJSEM Online).

**Table 2.** Cellular fatty acids of species of the genus \textit{Sphingomonas}.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<tbody>
<tr>
<td>(\text{C}_{14:0})</td>
<td>1.7</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>(\text{C}_{15:0})</td>
<td>0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{C}_{16:0})</td>
<td>12.8</td>
<td>9.2</td>
<td>11.4</td>
</tr>
<tr>
<td>(\text{C}_{18:0})</td>
<td>0.3</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>(\text{C}_{16:1}) (9c)</td>
<td>6.0</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>(\text{C}_{17:1}) (9c)</td>
<td>11.0</td>
<td></td>
<td>18.5</td>
</tr>
<tr>
<td>(\text{C}_{16:1}) (11c)</td>
<td>3.3</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>(\text{C}_{18:1}) (9c)</td>
<td>0.7</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>(\text{C}_{17:1}) (9c)</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{C}_{18:1}) (9c)</td>
<td>1.4</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>(\text{C}_{14:0}) 2-OH</td>
<td>7.4</td>
<td>8.3</td>
<td>8.6</td>
</tr>
<tr>
<td>(\text{C}_{15:0}) 2-OH</td>
<td>&lt;0.1</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>(\text{C}_{16:0}) 2-OH</td>
<td>&lt;0.1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Summed feature 4</td>
<td>9.3</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>Summed feature 7</td>
<td>55.6</td>
<td>51.6</td>
<td>54.3</td>
</tr>
</tbody>
</table>

Lipids were extracted by a modified Bligh–Dyer method (Fredrickson et al., 1986), followed by analysis in a mass spectrometer using fast atom bombardment (FAB-MS) ionization recorded in the negative mode of selected fractions as previously described by Abraham et al. (1997). Analysis of the individual phospholipids by MS revealed that \(\text{C}_{18:1}\) \(\text{C}_{16:1}\)-phosphatidyl-N,N-dimethylethylamine (743 Da), the corresponding choline (756 Da), \(\text{C}_{18:1}\) \(\text{C}_{18:1}\)-phosphatidic acid (700 Da), the corresponding -N,N-dimethylethylamine (771 Da) and corresponding choline (785 Da) were formed by nearly all members of the family \textit{Sphingomonadaceae} investigated. Interestingly, for the separated group of strains in the \textit{Sphingomonas} cluster, \textit{S. wittichii} RW1\(^T\) and strains K101\(^T\) and A175\(^T\), some lipids could be identified as occurring preferentially in these strains. These were \(\text{C}_{19:1}\) \(\text{C}_{16:0}\)-phosphatidic acid (688 Da) and -glycerol (762 Da), \(\text{C}_{18:1}\) \(\text{C}_{16:1}\)-N-methyl-ethanolamine (729 Da) and \(\text{C}_{18:1}\) \(\text{C}_{16:2}\)-phosphatidyl-N,N-dimethylethylamine (741 Da) (see Supplementary Table S1b in IJSEM Online).

On the basis of the 16S rRNA gene sequences, strains A175\(^T\) and K101\(^T\) were found to be only remotely related to other species of the genus \textit{Sphingomonas}. Although strain A175\(^T\) is more closely related to \textit{S. wittichii} RW1\(^T\) than to strain K101\(^T\) on the basis of 16S rRNA gene sequence comparisons, strain A175\(^T\) differs significantly from these sphingomonads in its polyamine pattern (see Supplementary Table S2 in IJSEM Online).

Our data do not support the notion of Takeuchi et al. (2001) that members of the genus \textit{Sphingomonas} and those of the genera \textit{Sphingobium}, \textit{Novosphingobium} and \textit{Sphingopyxis} can be discerned by the presence of sym-homospermidine and the absence of spermidine in \textit{Sphingomonas} species while species of the other three genera possess spermidine but not homospermidine (see Supplementary Table S2). Both polyamines were detected in \textit{S. wittichii} RW1\(^T\) and in strains A175\(^T\) and K101\(^T\), but were also detected in the type strains of \textit{Sphingomonas ascharolytica}, \textit{Sphingomonas paucimobilis}, \textit{Sphingomonas trueperi}, \textit{Sphingomonas abaci}, \textit{Sphingomonas panni}, \textit{Sphingomonas echinoideas} and \textit{Sphingomonas pituitosa} (Busse et al., 1999, 2005; Denner et al., 1999, 2001). However, all species of the genus \textit{Sphingomonas sensu stricto} are characterized by the predominant polyamine sym-homospermidine. These results show that species of the genus \textit{Sphingomonas} have mainly sym-homospermidine and some of them also produce minor quantities of spermidine. Species of the genera \textit{Sphingobium}, \textit{Novosphingobium} and \textit{Sphingopyxis} produce no sym-homospermidine. However, sym-homospermidine also characterizes species of the genus \textit{Sphingosinicella} (Geueke et al., 2007; Maruyama et al., 2006). Although strains K101\(^T\) and A175\(^T\) share most of the signature nucleotides proposed by Takeuchi et al. (2001) for the differentiation of members of the genus \textit{Sphingomonas sensu stricto} from related genera, these nucleotides are also found in species of the genus \textit{Sphingosinicella} (Geueke et al., 2007; Maruyama et al., 2001).
et al., 2006). In conclusion, neither the polyamine patterns nor the signature nucleotides allow the assignment of strains K101<sup>T</sup> and A175<sup>T</sup> to either of the two genera. The phylogenetic distance of the two novel strains to representatives of the genus Sphingosinicella and their close relatedness to S. wittichii, as well as differences in the signatures identified for members of the genus Sphingosinicella by Geuke et al. (2007) support the assignment of strains K101<sup>T</sup> and A175<sup>T</sup> to the genus Sphingomonas. Therefore, the names Sphingomonas fennica sp. nov. and Sphingomonas haloaromaticamans sp. nov. are proposed for strains K101<sup>T</sup> and A175<sup>T</sup>, respectively.

**Description of Sphingomonas haloaromaticamans sp. nov.**

Sphingomonas haloaromaticamans (ha.lo.ar.o.ma.tic.a.mans. N.L. n. haloaromaticum halaromatic, class of chemical compound; L. part. adj. amans loving; N.L. part. adj. haloaromaticamans loving halaromatics).

Cells are rod-shaped, 0.8–1.5 μm in length and 0.4–0.6 μm in diameter. Colonies are yellow-coloured. N-Acetyl-D-glucosamine, L-arabinose, p-arbutin, D-cellobiose, D-galactose, D-glucose, D-maltose, 2-D-melibiose, D-xylene, acetate, propionate, azelate, DL-3-hydroxybutyrate, DL-lactate, pyruvate, L-alanine, L-aspartate, L-histidine, L-leucine, L-proline and L-tryptophan are used as substrates, but not D-fructose, gluconate, D-mannose, L-rhamnose, sucrose, salicin, D-trehalose, maltitol, D-mannitol, D-sorbitol, cis-aconitate, adipate, 4-aminoxybutyrate, azelate, citrate, fumarate, DL-lactate, L-malate, L-ornithine, D-phenylalanine, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. pNP-phosphorylcholine, 2-deoxythymidine-5'-phosphate and L-alanine-pNA are hydrolysed, but aesculin, pNP-β-D-galactopyranoside, pNP-β-D-glucuronoside, pNP-α-D-glucopyranoside, pNP-β-D-glycopyranoside, bis-pNP-phosphate, pNP-phenylphosphonate, L-glutamate-γ-3-carboxy-pNA and L-proline-pNA are not hydrolysed. Polar lipids are phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglyco- and sphingoglycolipid. Major fatty acids are C18 : 1 (summed feature 7) and C16 : 0. Minor fatty acids are C14 : 0, C18 : 0, C16 : 1<sup>v</sup>, C17 : 1<sup>ω</sup>6c, C17 : 1<sup>ω</sup>8c and C18 : 1<sup>c</sup>. The major hydroxy-fatty acid is C14 : 0 2-OH together with minor amounts of C15 : 0 2-OH. The major polyamine is homospermidine; minor polyamines are putrescine, cadaverine, spermidine and spermine. Produces ubiquinone Q-10, but small amounts (2%) of Q-9 can also be detected. DNA G+C content of the type strain is 63.6 mol%. Grows between 10 and 37 °C with optimal growth between 20 and 30 °C. pH range for growth is 5–8, optimal pH is 7–8.

The type strain, K101<sup>T</sup> (=DSM 13665<sup>T</sup> = CCUG 53462<sup>T</sup>), was isolated from polychlorophenol-contaminated groundwater adjacent to a sawmill, Southern Finland, as a degrader of 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol. The environment from which the strain was originally isolated was cold (7–8 °C), oxygen-deficient, humic (4–23 mg dissolved organic carbon 1−1) and slightly acidic (pH 6–6.5).

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**References**


pentachlorophenol-degrading bacterial strains as *Sphingomonas chlorophenolica* sp. nov. *Syst Appl Microbiol* 18, 527–538.


