Description of Chlorophenol-Degrading *Pseudomonas* sp. Strains KF1<sup>T</sup>, KF3, and NKF1 as a New Species of the Genus *Sphingomonas*, *Sphingomonas subarctica* sp. nov.

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Bacteria that degrade xenobiotic compounds are usually studied for their biochemical activities, while the taxonomic descriptions of degrading strains are often given little or inadequate attention. As a consequence, many degrading bacteria have to be transferred to new species or genera once taxonomic characterizations are carried out (2, 4, 13, 35).

Previously, we reclassified four gram-negative pentachlorophenol-degrading strains as members of a new species of the genus *Sphingomonas*, *Sphingomonas chlorophenolica* (35), on the basis of chemotaxonomic and physiological data. Thus, all known bacterial strains that utilize pentachlorophenol as a sole source of carbon are placed in two genera; the genus *Alcaligenes* contains the gram-negative degraders, and the genus *Pseudomonas* contains the gram-positive degraders (4, 13, 14, 35). Bacteria that degrade other polychlorophenols are found in several genera, both gram negative and gram positive (14).

In this paper we describe the taxonomic properties of three gram-negative strains that can degrade polychlorinated phenols (38). We include detailed morphological, chemotaxonomic, physiological, and genetic data for these strains, which were originally described as *Pseudomonas saccharophila* strains (38), and we show that they belong to a new species, for which the name *Sphingomonas subarctica* is proposed. Taxonomic properties of several other bacteria with degradative properties, including *Beijerinckia* sp. strain B1 (recently reclassified as a *Sphingomonas yanoikuyae* strain by Khan et al. [26]), *Pseudomonas* sp. strain BN6, *Sphingomonas paucimobilis* EPA 505, and *Alcaligenes* sp. strain A175, were also studied and compared with *S. subarctica* properties.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** The bacterial strains used in this study are listed in Table 1. Polychlorophenol-degrading strains KF1<sup>T</sup> (T = type strain), KF3, and NKF1 were isolated from the biofilm of a continuous-flow fluidized-bed reactor inoculated with activated sludge and fed with a mixture of polychlorinated phenols (38). The bacteria were grown at 28°C in half-strength tryptone soy agar for 2 to 5 days unless indicated otherwise.

**Microscopy.** The morphology of living and stained cells was determined by light microscopy and electron microscopy. For negative staining 1 drop of a culture was placed on a copper grid coated with Pioloform and carbon and stained with 1% potassium phosphotungstic acid adjusted to pH 6.5 with potassium hydroxide. Thin sections were prepared and examined as described previously (58).

**Whole-cell fatty acids.** The whole-cell fatty acids of the bacteria were analyzed as methyl esters by gas chromatography (58). The aerobic library of the Microbial Identification System (MIDI, Inc., Newark, Del.) and Library version 3.8 were used to perform a comparative analysis.

**Sphingolipids.** Sphingolipids were extracted from 0.5 to 0.7 g (wet weight) of cells and were analyzed by using the method of Yabuuchi et al. (62), with the following modifications. For acid methanolysis 5 ml of 12 M HCl–methanol (1:3, vol/vol) was added to wet cells, the resulting suspension was heated for 2.5 to 3 h in a 100°C water bath, the fatty acid methyl esters were extracted three times with 2 ml of n-hexane-diethyl ether (1:1, vol/vol), and the water phase was made alkaline by dissolving three or four pellets of solid KOH in it. The sphingolines were extracted three times with 2 ml of n-hexane-diethyl ether (1:1, vol/vol), and the upper phases were collected for sphingolipid analysis. For mass spectrometric analyses the sphingolines were silylated as follows: the hexane-diethyl ether
extract was evaporated to dryness under a stream of N2, and 200 μl of bis(trimethylsilyl)trifluoroacetamide was added. The reaction mixture was heated at 70°C for 2 h, the reagent was evaporated under a stream of N2, and the residue was dissolved in 200 to 300 μl of hexane. The sphingosomes were analyzed with a gas-liquid chromatograph–mass spectrometer (model 5890A; Hewlett-Packard Co., Palo Alto, Calif.) equipped with a type HPS capillary column (Hewlett-Packard Co., Palo Alto, Calif.) and a mass selective detector (model HP 5970). The temperature was increased from 40 to 290°C at a rate of 8°C/min. DL-Dihydrosphingosine was dissolved in 200 to 300 μl of the quinone peaks were recorded at 200 to 600 nm. The identification was performed by using a HPLC apparatus equipped with two Waters model 510 pumps, a model UK6 injector, a type TCM column, and a JASCO model 821-FP spectrophotometer.

16S rDNA analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S ribosomal RNA (rDNA), and purification of the PCR products were carried out as described previously (40, 41). The purified PCR products were sequenced by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems Co., Foster City, Calif.) as described in the manufacturer’s protocol. Sequence reaction mixtures were electrophoresed by using a model 373A DNA sequencer (Applied Biosystems). The 16S rDNA sequences obtained were aligned manually with the sequences of representatives of the alpha subclass of the Proteobacteria. Pairwise evolutionary distances were computed by using the correction of Jukes and Cantor (21). The neighbor-joining method was used to reconstruct a phylogenetic tree from the distance matrices (44).

DNA-DNA reassociation studies. DNA was isolated by chromatography on hydroxyapatite by the procedure of Cashion et al. (6). DNA-DNA reassociation experiments were performed as described by De Ley et al. (8), with the modifications described by Huss et al. (19), by using a Gilford System model 2600 spectrophotometer equipped with a model 2527-R thermoprogrammer and plotter (Gilford Instruments, Oberlin, Ohio). Renaturation rates were computed with the TRANSFER.BAS program (20).

Oxidation of carbon sources. Oxidation of 95 different carbon sources was tested by using Biolog GN Microplates (Biolog, Inc., Hayward, Calif.) and an inoculum grown on Trypticase soy agar plates. Oxidation of individual carbon sources was detected indirectly by observing reduction of tetrazolium dye with Biolog GN MicroLog software after 24 h of incubation at 28°C. The auxillary program Miclast (MicroLog3 software) was used to construct dendrograms. Additional substrate utilization characteristics were determined by using API 20NE strips (Bio Mérieux, Marcy-l’Etoile, France).

Protein profiles. Protein profiles were determined from whole-cell protein extracts by reverse-phase high-performance liquid chromatography (HPLC), 200 μg of hydrophilized cells was crushed and extracted (34). A filtered sample (10 μl) was analyzed as described previously (35). The quinones were detected at 248 and 275 nm, and the spectra of the quinone peaks were recorded at 200 to 600 nm. The identification was confirmed with commercially available ubiquinones 9 and 10 (Sigma).

Base composition of DNA. Cells from 30 ml of a culture that had been grown in half-strength Trypticase soy medium for 15 to 24 h at 28°C with shaking at 180 rpm were collected, and DNA was isolated, digested, and analyzed as described previously (35).
FIG. 1. Negatively stained strain KF1<sup>T</sup>, KF3, and NKF1 cells. (a) Coccoid KF1<sup>T</sup> cells with flagella and thin fimbriae, which are longer than the cells. Bar = 1 μm. (b) Long helically twisted KF1<sup>T</sup> cell with a tapered end and many fimbriae. The surface of the cell is blebbing. Bar = 0.5 μm. (c) Ovoid cells of strain KF3, showing a flagellum and fimbriae. Bar = 1 μm. (d) Strain NK1 cell, showing the wrinkled cell surface and flagella. Bar = 0.5 μm.
TABLE 2. Whole-cell fatty acid compositions of polychlorophenol-degrading strains KFIT, KF3, and NKFl and reference strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>14:0 2OH</th>
<th>16:1 cis9</th>
<th>16:0</th>
<th>17:1 cis11</th>
<th>18:1 cis9</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>KFIT</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>61</td>
<td>6</td>
</tr>
<tr>
<td>KF3</td>
<td>9</td>
<td>14</td>
<td>10</td>
<td>1</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td>NKFl</td>
<td>8</td>
<td>14</td>
<td>10</td>
<td>2</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td>Alcaligenes</td>
<td>7</td>
<td>13</td>
<td>10</td>
<td>4</td>
<td>61</td>
<td>5</td>
</tr>
<tr>
<td>Beijerinckia</td>
<td>7</td>
<td>15</td>
<td>11</td>
<td>0</td>
<td>59</td>
<td>8</td>
</tr>
<tr>
<td>S. paucimobilis</td>
<td>7</td>
<td>25</td>
<td>11</td>
<td>0</td>
<td>53</td>
<td>1</td>
</tr>
<tr>
<td>S. sanguis</td>
<td>5</td>
<td>8</td>
<td>16</td>
<td>1</td>
<td>66</td>
<td>4</td>
</tr>
<tr>
<td>Sphingomonas</td>
<td>7</td>
<td>29</td>
<td>6</td>
<td>0</td>
<td>53</td>
<td>3</td>
</tr>
<tr>
<td>Sphingomonas</td>
<td>7</td>
<td>10</td>
<td>15</td>
<td>0</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>Sphingomonas</td>
<td>8</td>
<td>14</td>
<td>8</td>
<td>2</td>
<td>64</td>
<td>2</td>
</tr>
</tbody>
</table>

* Abbreviations: 14:0 2OH, 2-hydroxytetradecanoic acid (2-hydroxymyristic acid); 16:1 cis9, cis-9-hexadecenoic acid; 16:0, hexadecanoic acid; 17:1 cis11, cis-11-heptadecenoic acid; 18:1 cis9, octadecenoic acid (sum of cis-9-, trans-6-, and cis-11-octadecenoic acids).

RESULTS

Cell morphology and ultrastructure. Polychlorophenol-degrading strains KFIT, KF3, and NKFl formed yellow colonies within 1 to 2 days on half-strength Trypticase soy agar plates at 28°C. The colonies were slimy, especially when the organisms were grown at temperatures below the optimum temperature. The cells of these strains were gram-negative rods. Figures 1 and 2 show the morphology typical of cells of strains KFIT, KF3, and NKFl and of S. yanoikuyae DSM 7462T, which was used for comparison. In negatively stained preparations (Fig. 1) the shapes and wrinkled gram-negative surfaces of strain KFIT, KF3, and NKFl were observed. The cells of all four strains varied from small cocoid cells (Fig. 1a) that were 1 μm long to long helically twisted cells (Fig. 1b) or slime-coated chains consisting of several cells that were 3 μm long or longer. The widths of the cells varied between 0.7 and 1.1 μm, and the cells had rounded ends (Fig. 1a) or tapered ends (Fig. 1c). Thin fragile fimbriae, most of which were longer than the cells (Fig. 1a and b), were present in all four strains, together with flagella (Fig. 1a, c, and d). Chains of cells were observed frequently in thin sections (Fig. 2). Cells that were 1 to 3 μm long were held together by densely staining material (Fig. 2). This material easily detached or peeled off from the surfaces of the cells of strains KFIT, KF3, and NKFl (Fig. 2) and also from the surfaces of the cells of S. yanoikuyae DSM 7462T, which was used as a reference strain (Fig. 2d). The cells of reference strain DSM 7462T formed knobs and bubbles with laminated structures on the cell surface or between two adjacent cells. Reproduction occurred by asymmetric division of the mother cell which produced a shorter daughter cell at one pole of the mother cell (Fig. 2a).

Chemotaxonomic characterization. The whole-cell fatty acid compositions of the three polychlorophenol-degrading strains and selected reference strains are shown in Table 2. The dominant fatty acid in the polychlorophenol-degrading strains was octadecenoic acid, and the other significant fatty acids were 2-hydroxymyristic acid, cis-9-hexadecenoic acid, and hexadecenoic acid. An erroneous match with the three polychlorophenol-degrading strains in the whole-cell fatty acid database used was P. saccharophila; other chemotaxonomic data (see below) showed that this was not the correct diagnosis. Figure 3 shows the positions of the strains on a whole-cell fatty acid-based dendrogram; all three polychlorophenol-degrading strains are in the same cluster.

Sphingolipids were obtained from strains KFIT (Fig. 4), KF3, and NKFl, S. paucimobilis ATCC 29837T, and S. capsulata DSM 30196T. The trimethylsilyl derivatives of dihydrosphingosines were analyzed by gas chromatography-mass spectrometry, and it was found that all of the strains contained the same major dihydrosphingosines, d-18:0, d-20:1, and d-21:1 (number of carbon atoms:number of double bonds) (Fig. 4). Polychlorophenol-degrading strains KFIT, KF3, and NKFl contained d-18:1 (Fig. 4b) and d-19:1 (Fig. 4c) as minor dihydrosphingosines. The mass fragmentogram of a commercially available dihydrosphingosine with 18 carbon atoms (d-18:0) was identical to the mass fragmentograms of the d-18:0 dihydrosphingosines of strains KFIT, KF3, and NKFl, S. capsulata DSM 30196T, and S. paucimobilis ATCC 29837T. Burkholderia cepacia DSM 50180 and P. saccharophila DSM 654T were also analyzed and were found to contain no sphingolipid.

The respiratory quinones of the three polychlorophenol-degrading strains and reference strains were analyzed by HPLC. The ubiquinones of the three polychlorophenol-degrading strains eluted during HPLC with retention times (22.63 ± 0.05 min) identical to those of S. paucimobilis ATCC 29837T ubiquinone 10 and reference ubiquinone 10 purchased from Sigma. The following quinones of other reference strains had clearly different retention times: Stenotrophomonas maltophilia 5D ubiquinone 8 (retention time, 17.78 min), Pseudomonas putida G7 ubiquinone 9 (20.35 min), and Mycobacterium chlorophenolicum CG-1 menaquinone MK-9H2 (24.33 min).

TABLE 3. Cellular polyamine contents of strains KFIT, KF3, and NKFl

<table>
<thead>
<tr>
<th>Strain</th>
<th>DAP</th>
<th>PUT</th>
<th>CAD</th>
<th>TYR</th>
<th>NSPD</th>
<th>SPD</th>
<th>SPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>KFIT</td>
<td>82.1</td>
<td>4.6</td>
<td>2.3</td>
<td>0.7</td>
<td>0.2</td>
<td>Tr</td>
<td>4.6</td>
</tr>
<tr>
<td>KF3</td>
<td>87.2</td>
<td>5.0</td>
<td>0.2</td>
<td>Tr</td>
<td>0.2</td>
<td>Tr</td>
<td>3.5</td>
</tr>
<tr>
<td>NKFl</td>
<td>87.2</td>
<td>5.0</td>
<td>0.2</td>
<td>Tr</td>
<td>0.2</td>
<td>Tr</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Abbreviations: DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; TYR, tyramine; NSPD, sym- norspermidine; SPD, spermidine; SPM, spermine.
The G+C content of the DNA of strain KFIT was 66.2 ± 1 mol%, which falls within the range of values found for members of the genus *Sphingomonas* (62 to 67 mol%) (62).

The polyamine patterns of strains KFIT, KF3, and NKF1 were dominated by the presence of spermidine. In addition, low amounts of spermine and traces of 1,3-diaminopropane, putrescine, cadaverine, tyramine, and *sym*-norspermidine were detected (Table 3).

**TABLE 5. Levels of homology for the chromosomal DNAs of strain KFIT and *Sphingomonas* reference strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>% Homology with DNA from:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KFIT&lt;sup&gt;T&lt;/sup&gt;</td>
</tr>
<tr>
<td>KFIT&lt;sup&gt;T&lt;/sup&gt;</td>
<td>37.2</td>
</tr>
<tr>
<td><em>S. paucimobilis</em> ATCC 29837&lt;sup&gt;T&lt;/sup&gt;</td>
<td>37.2</td>
</tr>
<tr>
<td><em>S. capsulata</em> DSM 30196&lt;sup&gt;T&lt;/sup&gt;</td>
<td>39.8</td>
</tr>
<tr>
<td><em>S. yanoikuyae</em> DSM 7462&lt;sup&gt;T&lt;/sup&gt;</td>
<td>32.3</td>
</tr>
<tr>
<td><em>S. chlorophenolica</em> ATCC 29837&lt;sup&gt;T&lt;/sup&gt;</td>
<td>35.3</td>
</tr>
<tr>
<td><em>S. chlorophenolica</em> ATCC 33790&lt;sup&gt;T&lt;/sup&gt;</td>
<td>25.5</td>
</tr>
<tr>
<td><em>S. chlorophenolica</em> ATCC 39723</td>
<td>33.1</td>
</tr>
<tr>
<td><em>S. chlorophenolica</em> RA2</td>
<td>30.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not determined.
FIG. 2. Thin sections of strains KF1<sup>T</sup> and NKFl and S. yanoikyae DSM 7462<sup>T</sup>. (a) Long filamentous cell of strain NKFl (middle) with a small polarly dividing daughter cell. Bar = 0.5 μm. (b) Newborn NKFl cells held together by densely staining multilayered material at the point of division. Bar = 0.5 μm. (c) Large masses of densely staining material polarly attached to small cells, possibly at recent sites of cell division. Bar = 0.5 μm. (d) High magnification of S. yanoikyae DSM 7462<sup>T</sup> cells with piles of multilayered material similar to the material observed in panels a through c. The material blebs or rolls off the cell surface at the cell division point and at other sites of the cell surface. Bar = 0.2 μm.
dendrogram reconstructed from distance matrices is shown in Fig. 5. The corresponding 16S rDNA similarity values are shown in Table 4. All nonambiguous base positions between position 30 and position 1444 (Escherichia coli numbering) were included in the phylogenetic analysis. The 16S rDNA sequences of polychlorophenol-degrading strains KFIT, KF3, and NKFl were found to be identical (Table 4). A comparison of the 16S rDNA sequences of strains KFIT, KF3, and NKFl with the sequences of the Sphingomonas reference strains (Fig. 5) showed that strains KFIT, KF3, and NKFl are the closest relatives of S. rosa and S. capsulata. Polychlorophenol-degrading strains KFIT, KF3, and NKFl represent a distinct lineage that exhibits 96.1 and 95.9% 16S rDNA sequence similarity to S. capsulata and S. rosa, respectively. The 16S rDNA sequences of these strains were also compared with the sequences of the type strains of three recently described Sphingomonas species, Sphingomonas mali, Sphingomonas pruni, and Sphingomonas asaccharolytica, by using partial 16S rDNA sequences of these strains consisting of a 604-bp 16S rDNA fragment extending from nucleotide 227 to nucleotide 501, from nucleotide 720 to nucleotide 894, and from nucleotide 1180 to nucleotide 1383 (E. coli numbering). A comparison of the strain KFIT, KF3, and NKFl sequences with the sequences of S. mali, S. pruni, and S. asaccharolytica gave levels of similarity of 95.5 to 96.6% for the partial 16S rDNA sequences available (data not shown).

The levels of relatedness between the DNA of strain KFIT and the DNAs of S. paucimobilis ATCC 29837T, S. capsulata DSM 30196T, S. yanoikuyae DSM 7462T, and S. chlorophenolica ATCC 33790T, ATCC 39723, SR3, and RA2 were less than 40% (Table 5), which confirmed that strains KFIT, KF3, and NKFl are members of a separate species. The levels of DNA-DNA relatedness for the chlorohenol-degrading organisms S. chlorophenolica ATCC 33790T, ATCC 39723, SR3, and RA2 ranged from 67 to 97%, but the levels of DNA-DNA relatedness between these strains and strains KFIT, KF3, and NKFl were less than 40%. This confirmed our previous description of the four S. chlorophenolica strains as members of one species (35).

Metabolic properties. Strains KFIT, KF3, and NKFl oxidized about 50 of the 95 different carbon sources tested with the Biolog identification system; these results were similar to the results obtained with S. paucimobilis ATCC 29837T and S. capsulata DSM 30196T, which were used as reference organisms. The substrates oxidized by strains KFIT, KF3, and NKFl in the Biolog system after 24 h were α-cyclodextrin, dextrin, glycogen, Tween 40, Tween 80, N-acetyl-d-galactosamine, L-arabinose, D-fructose, d-galactose, gentiobiose, α-D-glucose, maltose, L-rhamnose, D-trehalose, methylpyruvate, monomethylsuccinate, acetic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, α-keto-butyric acid, α-keto-valeric acid, d,L-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, alanaminamide, D-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-leucine, L-proline, L-serine, L-threonine, and urocanic acid. Strains KFIT, KF3, and NKFl were esculin and p-nitrophenyl-β-D-galactopyranoside positive in the API 20NE test after 48 h and assimilated glucose, arabinose, N-acetylglucosamine, maltose, malate, and citrate. Polychlorophenol-degrading strains KFIT, KF3, and NKFl belonged to a cluster separated from the other Sphingomonas strains on the dendrogram derived from the Biolog system data based on oxidation of 95 different carbon sources (Fig. 6).

The protein profile dendrogram (Fig. 7) showed that polychlorophenol-degrading strains KFIT, KF3, and NKFl were more similar to each other than to reference organisms S. capsulata DSM 30196T, S. paucimobilis ATCC 29837T, and P. saccharophila DSM 654T or to the pentachlorophenol-degrading organisms S. chlorophenolica ATCC 33790T, ATCC 39723, RA2, and SR3.

The results of the cellular lipid component analysis and the polyamine patterns showed that polychlorophenol-degrading strains KFIT, KF3, and NKFl belong to a sphingolipid-containing taxon which has ubiquinone 10 as its major respiratory quinone, has a G+C content of 66 ± 1 mol%, and has spermidine as its dominant polyamine (Table 3) and that the cellular fatty acid compositions of these strains are very similar (Table 2). The phylogenetic analysis of the 16S rDNA sequences showed that the level of sequence similarity for strains KFIT, KF3, and NKFl was 100% and that the levels of similarity for the complete 16S rDNA sequence between these strains and other taxa were less than 96.1% (Table 4).

The polychlorophenol-degrading Sphingomonas strains formed two distinct clusters on the dendrograms derived from analyses of 16S rDNA sequences, whole-cell proteins, and the ability to oxidize carbon sources. S. chlorophenolica ATCC 33790T, ATCC 39723, RA2, and SR3 fall into one cluster, and S. subarcuata KFIT, KF3, and NKFl fall into another. The data obtained for the other xenobiotic compound-degrading strains included in this study, Alcaligenes sp. strain A175, Beijerinckia sp. strain B1 (DSM 6900), and Pseudomonas sp. strain BN6 (DSM 6383), show that these strains are related to the genus Sphingomonas. Beijerinckia sp. strain B1 exhibited 99.4% similarity over the complete 16S rDNA sequence with the type strain of S. yanoikuyae. Also, the results of the fatty acid composition analysis (Fig. 3) and the Biolog profile analysis (Fig. 6) confirmed that strain B1 should be considered a strain of S. yanoikuyae. S. paucimobilis EPA 505 (DSM 7526) is, on the basis of phylogenetic and chemotaxonomic criteria, much more closely related to S. chlorophenolica (level of similarity over the complete 16S rDNA sequence, 97.0%) than to S. paucimobilis (level of similarity, 92.8%) (Table 4). Both Alcaligenes sp. strain A175 and Pseudomonas sp. strain BN6 (DSM 6383) were located at separate positions on the dendrograms based on data derived from the 16S rDNA analysis and the analysis of the carbon sources oxidized, indicating that they may represent new species. Sphingomonas sp. strains SS3 (DSM 6432), RW1 (DSM 6014), and HH69 (DSM 7135)
FIG. 4. (a) Mass fragmentogram of the d-18:0 sphingosine peak of strain KF1T. (b through e) Structural formulas of other trimethylsilyl (TMS) derivatives of sphingosines of strains KF1T, KF3, and NKF1.

DISCUSSION

The genus *Sphingomonas* was created by Yabuuchi et al. (62) for gram-negative, aerobic, rod-shaped organisms which grow as yellow to whitish brown colonies. The members of the genus *Sphingomonas* contain sphingolipids and do not contain lipopolysaccharide; ubiquinone 10 is the main respiratory quinone of these organisms, the major cellular fatty acids are octadecanoate, 2-hydroxymyristate, cis-9-hexadecenoate, and hexadecanoate, and the DNA G+C content is 62 to 67 mol%. Polychlorophenol-degrading strains KF1T, KF3, and NKF1, which were described previously as strains of *P. saccharophila* (38), have these characteristics. The other sphingolipid-containing genera, *Erythrobacter* (49), *Sphingobacterium* (61), *Porphyrobacter* (11), and *Zymomonas* (54), differ from the genus *Sphingomonas* by having different G+C contents and chemotaxonomic characteristics, such as different whole-cell fatty acid compositions.

The dendrograms derived from analyses of different geno-
Sphingomonas species and isolates KF1\(^7\), KF3, and NKF1 are in the same range as the levels of similarity obtained for the sym-homospermidine-containing species. As it has been demonstrated that polyamine patterns are relatively conserved characteristics, the different polyamine patterns confirm the conclusion based on 16S rDNA sequence data that strains KF1\(^7\), KF3, and NKF1 are not related to the sym-homospermidine-containing Sphingomonas species at the species level. If the fact that the levels of 16S rDNA sequence similarity (Table 4) between the spermidine-containing Sphingomonas species and isolates KF1\(^7\), KF3, and NKF1 are in the same range as the levels of similarity obtained for the sym-homospermidine-containing species is taken into account, it is clear that these three isolates cannot be related at the species level to any species belonging to the spermidine-containing group.

We found that the major dihydrosphingosines of polychlorophenol-degrading strains KF1\(^7\), KF3, and NKF1 (d-18:0, d-20:1, and d-21:1) were similar to the major dihydrosping-
Sphingomonas subarctica sp. nov.

**Description of Sphingomonas subarctica sp. nov.**

*S. subarctica* (sub.ar'ci.ca. M.L. adj. *subarctica*, below the arctic, because the organism was isolated from a subarctic area, Finland) cells are gram-negative, non-spore-forming, fimbriated rods, often with a flagellum. The cell size is 0.5 to 3 by 0.5 to 1 μm. The cells produce visible, circular, smooth, yellow colonies on Trypticase soy agar in 1 to 2 days. Mesopholic and aerobic. Esculin and ρ-nitrophenyl-β-D-galactopyranoside positive. Utilizes 2,4,6-tri- and 2,3,4,6-tetrachlorophenols and oxidizes α-cyclohexidrin, dextrin, glycerogen, Tween 40, Tween 80, N-acetyl-D-galactosamin, L-arabinose, D-fructose, D-galactose, gentiobiode, α-D-glucose, maltose, D-xylose, D-xylose, maltose, malate, and uronate. The main dihydrophosphoginoses of *S. paucimobilis*, *S. capsulata*, *S. yanoikuyae*, and *S. adhaesiva* (56, 62). The major dihydrophosphoginoses of *S. san- guis*, *S. macrogolobulatus*, and *S. terrae* have been reported to be d-18:0, d-19:1, and d-20:1 (55). The most abundant sphingogisone in the genus *Sphingobacterium* is a branched-chain methyl-

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**Percentage similarity**

<table>
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<th>Strain</th>
<th>50</th>
<th>60</th>
<th>70</th>
<th>80</th>
<th>90</th>
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<tbody>
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<td>KF3</td>
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<tr>
<td>NKF1</td>
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</table>

**FIG. 7.** Protein profile dendrogram derived from whole-cell protein patterns, showing the clustering of strains KF1, KF3, and NKF1 and reference strains.
from the other known chlorophenol-degrading *Sphingomonas* species, *S. chlorophenolica*, phylogenetically, by its more rapid growth rate, and by exhibiting clear positive reactions for assimilation of glucose, arabinose, *N*-acyetylglucosamine, maltose, and citrate in API 20NE tests. *S. subarctica* KF1 (HANNI 2110), KF3 (HANNI 2111), and NKF1 (HANNI 2112) degrade 2,4,6-tri- and 2,3,4,6-tetrachlorophenols but not pentachlorophenol. The type strain is strain KFI (HANNI 2110).

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**REFERENCES**

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SPHINGOMONAS SUBARCTICA SP. NOV.


