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

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Gram-negative polychlorophenol-degrading bacterial strains KF1^T (T = type strain), KF3, and NKF1, which were described previously as *Pseudomonas saccharophila* strains, were studied by chemotaxonomic, genetic, and physiological methods and by electron microscopy and compared with selected xenobiotic compound-degrading bacteria. These strains contained sphingolipids with d-18:0, d-20:1, and d-21:1 as the main dihydrosphingosines, ubiquinone 10 as the main respiratory quinone, and spermidine as the major polyamine, and the DNA G+C content was 66 mol%. The cellular fatty acids included about 60% octadecenoic acid, 9% 2-hydroxymyristic acid, 14% *cis*-9-hexadecenoic acid, and 10% hexadecanoic acid. These strains exhibited less than 97% 16S ribosomal DNA sequence similarity to all of the other taxa studied. In the DNA-DNA reassociation studies the highest levels of reassociation between these strains and previously described species were less than 40%. Thin sections of cells of strains KF1^T, KF3, and NKF1 were examined by electron microscopy, and the results showed that the cells had peculiar concentrically arranged layered membranous blebs that extruded from the outer membrane, especially at the cell division points. On the basis of the results of this study, polychlorophenol-degrading strains KF1^T, KF3, and NKF1 are considered members of a new species of the genus *Sphingomonas*, *Sphingomonas subarctica*. The polycyclic aromatic hydrocarbon-degrading organism *Sphingomonas paucimobilis* EPA 505 was closely related to *Sphingomonas chlorophenolica* as determined by chemotaxonomic, phylogenetic, and physiological criteria. The xenobiotic compound degraders *Alcaligenes* sp. strain A175 and *Pseudomonas* sp. strain BN6 were identified as members of species of the genus *Sphingomonas*.

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 *Sphingomonas* contains the gram-negative degraders, and the genus *Mycobacterium* 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.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study are listed in Table 1. Polychlorophenol-degrading strains KF1^T (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 Trypticase 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 sphingosines were extracted three times with 2 ml of hexane-diethyl ether (1:1, vol/vol), and the upper phases were collected for sphingosine analysis. For mass spectrometric analyses the sphingosines were silylated as follows: the hexane-diethyl ether

TABLE 1. Strains used in this study

Name as received	Culture collection no.[a]	Source[a]	Reference(s)
Pseudomonas saccharophila KF1^T	HAMBI 2110^T	R. Herwig	38
Pseudomonas saccharophila KF3	HAMBI 2111	R. Herwig	38
Pseudomonas saccharophila NKF1	HAMBI 2112	R. Herwig	38
Reference strains			
Alcaligenes sp. strain A175		G. Schraa	47
Beijerinckia sp. strain B1[b]	DSM 6900	C. Cerniglia	12
Burkholderia cepacia	DSM 50180	DSM	
Mycobacterium chlorophenolicum CG-1		Our laboratory	13
Pseudomonas putida G7		C. Cerniglia	
Pseudomonas saccharophila	DSM 654^T	DSM	
Pseudomonas sp. strain BN6[c]	DSM 6383	DSM	36
Sphingomonas adhaesiva	DSM 7418^T	DSM	62
Sphingomonas capsulata	DSM 30196^T	DSM	62
Sphingomonas chlorophenolica	ATCC 33790^T	R. Müller	10, 35
Sphingomonas chlorophenolica SR3		S. Resnick	35, 42
Sphingomonas chlorophenolica	ATCC 39723	R. Crawford	35, 43
Sphingomonas chlorophenolica RA2	DSM 8671	S. Schmidt	35, 39
Sphingomonas macrogoltabidus	IFO 15033^T	F. Kawai	55
Sphingomonas macrogoltabidus 103		F. Kawai	55
Sphingomonas parapaucimobilis	DSM 7463^T	DSM	62
Sphingomonas paucimobilis	ATCC 29837^T	ATCC	62
Sphingomonas paucimobilis EPA 505[d]	DSM 7526	DSM	32
Sphingomonas sanguis	IFO 13937^T	IFO	55
Sphingomonas terrae	IFO 15098^T	F. Kawai	55
Sphingomonas terrae 411		F. Kawai	55
Sphingomonas yanoikuyae	DSM 7462^T	DSM	62
Sphingomonas sp. strain HH69-3	DSM 7135	DSM	17
Sphingomonas sp. strain RW1	DSM 6014	DSM	60
Sphingomonas sp. strain SS3	DSM 6432	DSM	46
Stenotrophomonas maltophilia 5D		Our laboratory	

[a] Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; HAMBI, Culture Collection of the Department of Applied Chemistry and Microbiology, Helsinki, Finland; IFO, Institute for Fermentation, Osaka, Japan.

[b] *Beijerinckia* sp. strain B1 was recently reclassified as *S. yanoikuyae* B1 by Khan et al. (26).

[c] Strain BN6 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as a *Sphingomonas* sp. strain.

[d] *Pseudomonas paucimobilis* was renamed *S. paucimobilis* by Yabuuchi et al. (62).

extract was evaporated to dryness under a stream of N_2, 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 N_2, and the residue was dissolved in 200 to 300 μl of hexane. The sphingosines were analyzed with a gas-liquid chromatograph–mass spectrometer (model 5890A; Hewlett-Packard Co., Palo Alto, Calif.) equipped with a type HP5 capillary column (Hewlett-Packard Co.) 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 (Sigma Chemical Co., St. Louis, Mo.) was used as the reference compound.

Respiratory quinones. To analyze respiratory quinones by reverse-phase high-performance liquid chromatography (HPLC), 200 mg of lyophilized 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, extracted, digested to the nucleoside level, and analyzed as described previously (35).

Analysis of polyamines. Polyamines were extracted and analyzed as described previously (5, 45) 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 spectrofluorometer.

16S rDNA analysis. Genomic DNA extraction, PCR-mediated amplification of the 16S ribosomal DNA (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 MicroLog3 software after 24 h of incubation at 28°C. The ancillary program Mlclust (MicroLog3 software) was used to construct dendrograms. Additional substrate utilization characteristics were determined by using API 20NE strips (Bio Mérieux, Marcy-l'Étoile, France).

Protein profiles. Protein profiles were determined from whole-cell protein patterns as described previously (16, 35). The results were used to construct an unweighted pair group with mathematical average dendrogram (51).

Nucleotide sequence accession numbers. The 16S rDNA sequences determined by us have been deposited in the EMBL database (Cambridge, United Kingdom) under the following accession numbers: KF1^T, X94102; KF3, X94103; NKF1, X94104; *Beijerinckia* sp. strain B1, X94099; *Pseudomonas* sp. strain BN6, X94098; *Alcaligenes* sp. strain A175, X94101; and *S. paucimobilis* EPA 505, X94100. The sequences of the reference strains were obtained from the Ribosomal Database Project (29). The nucleotide sequence accession numbers for the reference strains are as follows: *Sphingomonas adhaesiva* JCM 7370^T, X72720; *Sphingomonas capsulata* ATCC 14666^T, M59296; *Sphingomonas rosa* IFO 15208^T, D13945; *Blastobacter natatorius* ATCC 35951^T, X73043; *Rhizomonas suberifaciens* IFO 15211^T, D13737; *Sphingomonas macrogoltabidus* IFO 15033^T, D13723; *Sphingomonas terrae* IFO 15098^T, D13727; *S. yanoikuyae* IFO 15102^T, X72725; *S. paucimobilis* ATCC 10829^T (previously the type strain of *Flavobac-*

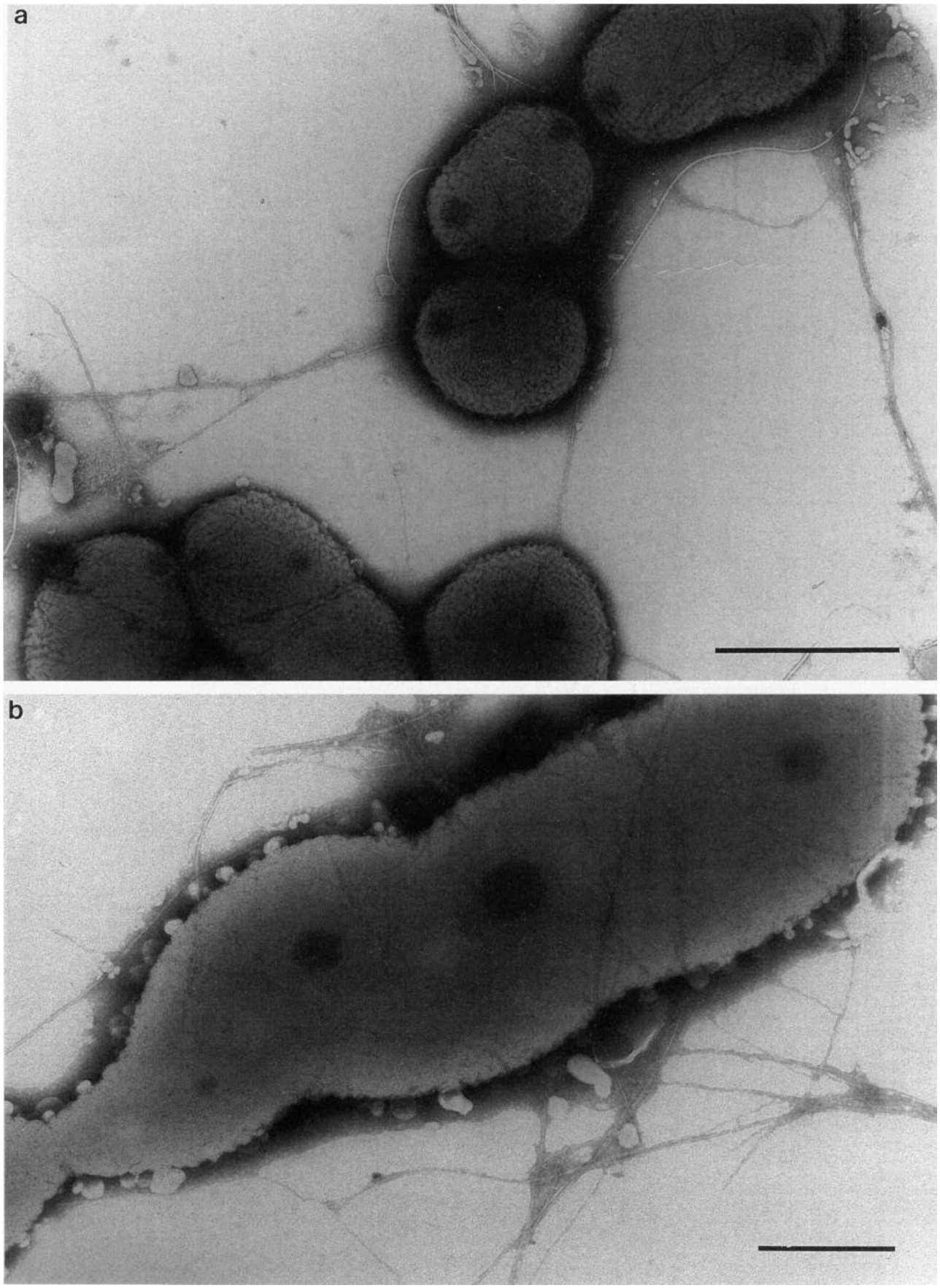


FIG. 1. Negatively stained strain KF1^T, KF3, and NKF1 cells. (a) Coccoid KF1^T cells with flagella and thin fimbriae, which are longer than the cells. Bar = 1 μm. (b) Long helically twisted KF1^T cell with a tapered end and many fimbriae. The surface of the cell is blebbing. Bar = 0.5 μm. (c) Ovoid cells of strain KF3, showing a flagellum and fimbriae. Bar = 1 μm. (d) Strain NFK1 cell, showing the wrinkled cell surface and flagella. Bar = 0.5 μm.

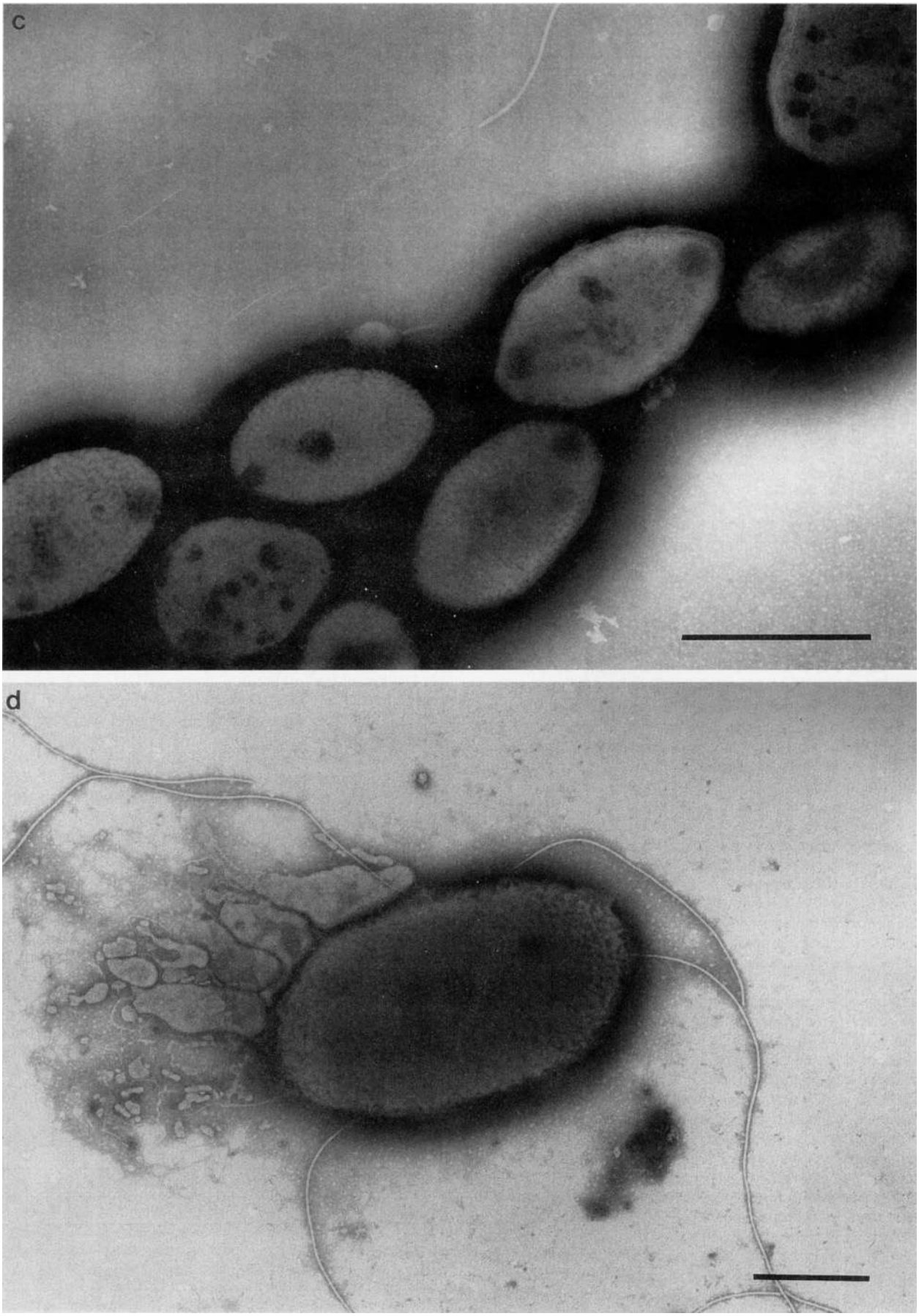


FIG. 1—*Continued.*

TABLE 2. Whole-cell fatty acid compositions of polychlorophenol-degrading strains KF1^T, KF3, and NKF1 and reference strains

Strain	Fatty acid content (% [wt/wt], ±2%)[a]					
	14:0 2OH	16:1 *cis*9	16:0	17:1 *cis*11	18:1 F#7	Other
KF1^T	8	14	10	1	61	6
KF3	9	14	10	1	60	6
NKF1	8	14	10	2	61	5
Alcaligenes sp. strain A175	7	13	10	4	61	5
Beijerinckia sp. strain B1	7	15	11	0	59	8
Pseudomonas sp. strain BN6	6	25	11	0	53	5
S. paucimobilis EPA 505	7	8	7	3	68	7
S. sanguis IFO 13937^T	5	8	16	1	66	4
Sphingomonas sp. strain HH69-3	7	29	6	0	53	5
Sphingomonas sp. strain RW1	7	10	15	0	60	8
Sphingomonas sp. strain SS3	8	14	8	2	64	2

[a] Abbreviations: 14:0 2OH, 2-hydroxytetradecanoic acid (2-hydroxymyristic acid); 16:1 *cis*9, *cis*-9-hexadecenoic acid; 16:0, hexadecanoic acid; 17:1 *cis*11, *cis*-11-heptadecenoic acid; 18:1 F#7, octadecenoic acid (sum of *cis*-9-, *trans*-6-, and *cis*-11-octadecenoic acids).

terium devorans), X72722; *S. chlorophenolica* ATCC 33790^T, X87161; *S. chlorophenolica* ATCC 39723, X87163; *Zymomonas mobilis* subsp. *mobilis* ATCC 10988^T, ARB-528391E2; *Sphingomonas sanguis* IFO 13937^T, D13726; *Sphingomonas parapaucimobilis* IFO 15100^T, X72721; *Caulobacter subvibrioides* CB81, M83797; *Blastobacter* sp. strain BF14, Z23157; *Erythrobacter longus* ATCC 33941^T, M59062; *Porphyrobacter neustonensis* ACM 2844^T, M96745; and *Brevundimonas diminuta* ATCC 11568^T, M59064.

RESULTS

Cell morphology and ultrastructure. Polychlorophenol-degrading strains KF1^T, KF3, and NKF1 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 KF1^T, KF3, and NKF1 and of *S. yanoikuyae* DSM 7462^T, which was used for comparison. In negatively stained preparations (Fig. 1) the shapes and wrinkled gram-negative surfaces of strain KF1^T, KF3, and NKF1 cells were observed. The cells of all four strains varied from small coccoid 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 KF1^T, KF3, and NKF1 (Fig. 2) and also from the surfaces of the cells of *S. yanoikuyae* DSM 7462^T, which was used as a reference strain (Fig. 2d). The cells of reference strain DSM 7462^T 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 hexadecanoic acid. An erroneous match with the three polychlorophenol-degrading strains in the whole-cell fatty acid library 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 KF1^T (Fig. 4), KF3, and NKF1, *S. paucimobilis* ATCC 29837^T, and *S. capsulata* DSM 30196^T. 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 KF1^T, KF3, and NKF1 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 KF1^T, KF3, and NKF1, *S. capsulata* DSM 30196^T, and *S. paucimobilis* ATCC 29837^T. *Burkholderia cepacia* DSM 50180 and *P. saccharophila* DSM 654^T 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 29837^T 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-9H$_2$ (24.33 min).

TABLE 3. Cellular polyamine contents of strains KF1^T, KF3, and NKF1

Strain	Polyamine content (μmol/g [dry wt])[a]						
	DAP	PUT	CAD	TYR	NSPD	SPD	SPM
KF1^T	0.3	0.7	0.2	0.2	Tr	72.1	4.6
KF3	0.3	0.2	Tr	0.2	Tr	67.2	5.0
NKF1	0.2	0.2	Tr	0.2	Tr	63.6	3.5

[a] Abbreviations: DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; TYR, tyramine; NSPD, *sym*-norspermidine; SPD, spermidine; SPM, spermine.

TABLE 4. Levels of 16S rDNA similarity for strains KF1^T, KF3, and NKF1 and related taxa

Strain[a]	% Similarity																					
	Strain KF1^T	Strain KF3	Strain NKF1	*Sphingomonas rosa* IFO 15208^T	*Sphingomonas capsulata* ATCC 14666^T	*Blastobacter natatorius* ATCC 35951^T	*Rhizomonas suberifaciens* IFO 15211^T	*Sphingomonas macrogoltabidus* IFO 15033^T	*Sphingomonas terrae* IFO 14098^T	*Sphingomonas yanoikuyae* DSM 6900	*Sphingomonas yanoikuyae* IFO 15102^T	*Sphingomonas paucimobilis* DSM 7256	*Sphingomonas chlorophenolica* ATCC 33790^T	*Sphingomonas chlorophenolica* ATCC 39723	*Pseudomonas* sp. strain DSM 6383	*Sphingomonas adhaesiva* JCM 7370^T	*Sphingomonas sanguis* IFO 13937^T	*Sphingomonas parapaucimobilis* IFO 15100^T	*Sphingomonas paucimobilis* ATCC 10829	*Caulobacter subvibrioides* CB81	*Blastobacter* sp. strain BF14	*Alcaligenes* sp. strain A175
Strain KF3	100																					
Strain NKF1	100	100																				
Sphingomonas rosa IFO 15208^T	95.9	95.9	95.9																			
Sphingomonas capsulata ATCC 14666^T	96.1	96.1	96.1	96.3																		
Blastobacter natatorius ATCC 35951^T	92.4	92.4	92.4	91.9	92.2																	
Rhizomonas suberifaciens IFO 15211^T	94.2	94.2	94.2	95.1	93.6	92.9																
Sphingomonas macrogoltabidus IFO 15033^T	95.4	95.4	95.4	94.3	94.2	92.3	94.9															
Sphingomonas terrae IFO 15098^T	94.8	94.8	94.8	94.4	93.5	91.8	95.3	96.5														
Sphingomonas yanoikuyae DSM 6900	94.8	94.8	94.8	94.1	93.7	92.1	95.5	94.2	94.6													
Sphingomonas yanoikuyae IFO 15102^T	94.7	94.7	94.7	93.7	93.6	92.3	95.5	94.3	94.6	99.5												
Sphingomonas paucimobilis DSM 7526	93.3	93.3	93.3	93.0	92.6	91.4	94.8	93.6	93.9	95.6	95.3											
Sphingomonas chlorophenolica ATCC 33790^T	93.4	93.4	93.4	93.3	91.9	91.2	94.5	93.2	93.3	96.8	96.6	97.0										
Sphingomonas chlorophenolica ATCC 39723	92.8	92.8	92.8	92.5	91.7	90.2	93.2	92.7	92.2	95.9	95.4	96.5	97.6									
Pseudomonas sp. strain DSM 6383	94.2	94.2	94.2	94.3	93.0	91.4	95.3	94.4	94.2	96.0	95.7	95.8	95.5	94.9								
Sphingomonas adhaesiva JCM 7370^T	93.1	93.1	93.1	94.0	93.2	90.9	93.7	92.7	92.9	93.9	93.6	93.1	93.1	92.9	93.7							
Sphingomonas sanguis IFO 13937^T	92.7	92.7	92.7	93.3	92.8	90.9	93.5	92.9	93.2	93.9	93.6	93.3	93.0	92.5	93.5	97.1						
Sphingomonas parapaucimobilis IFO 15100^T	92.8	92.8	92.8	93.5	93.1	90.8	93.7	92.9	93.1	94.1	93.7	93.6	93.3	93.0	93.7	96.7	99.1					
Sphingomonas paucimobilis ATCC 10829	92.9	92.9	92.9	93.3	92.4	90.5	93.3	93.1	93.1	93.6	93.3	92.8	92.5	92.6	93.4	96.5	98.1	98.0				
Caulobacter subvibrioides CB81	93.7	93.7	93.7	94.5	93.4	92.5	95.4	93.6	94.1	94.8	94.8	93.6	93.9	93.1	94.4	97.2	96.6	96.8	96.2			
Blastobacter sp. strain BF14	91.5	91.5	91.5	91.7	91.6	90.0	93.2	92.3	91.8	92.9	92.7	91.7	92.5	91.7	92.3	95.2	94.8	94.6	94.2	95.6		
Alcaligenes sp. strain A175	92.8	92.8	92.8	91.7	90.8	93.4	93.1	93.7	94.2	93.9	94.5	94.4	93.5	92.9	93.2	93.7	93.7	93.5	93.6	92.0		
Zymomonas mobilis ATCC 10988^T	91.7	91.7	91.7	91.7	90.7	88.0	91.2	92.3	91.7	91.7	91.5	91.4	91.2	91.2	91.5	91.0	91.0	90.7	91.5	91.1	89.8	92.5

[a] *Beijerinckia* sp. strain B1 has been renamed *S. yanoikuyae* DSM 6900, and *S. paucimobilis* EPA 505 and *Pseudomonas* sp. strain BN6 have been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as strains DSM 7526 and DSM 6383, respectively.

The G+C content of the DNA of strain KF1^T was 66 ± 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 KF1^T, 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).

Phylogenetic analysis. Almost-complete 16S rDNA sequences of strains KF1^T, KF3, and NKF1, *Alcaligenes* sp. strain A175, *Beijerinckia* sp. strain B1, *Pseudomonas* sp. strain BN6, and *S. paucimobilis* EPA 505 were determined. A phylogenetic

TABLE 5. Levels of homology for the chromosomal DNAs of strain KF1^T and *Sphingomonas* reference strains

Strain	% Homology with DNA from:							
	KF1^T	ATCC 29837^T	DSM 30196^T	DSM 7462^T	ATCC 33790^T	SR3	ATCC 39723	RA2
KF1^T		37.2	39.8	32.3	35.3	25.5	33.1	30.6
S. paucimobilis ATCC 29837^T	37.2		ND[a]	ND	28.8	ND	33.4	ND
S. capsulata DSM 30196^T	39.8	ND		ND	25.3	ND	24.7	ND
S. yanoikuyae DSM 7462^T	32.3	ND	ND		36.4	40.0	28.6	ND
S. chlorophenolica ATCC 33790^T	35.3	28.8	25.3	36.4		90.3	70.3	66.5
S. chlorophenolica SR3	25.5	ND	ND	40.0	90.3		70.1	76.0
S. chlorophenolica ATCC 39723	33.1	33.4	24.7	28.6	70.3	70.1		95.5
S. chlorophenolica RA2	30.6	ND	ND	ND	66.5	76.0	95.5	

[a] ND, not determined.

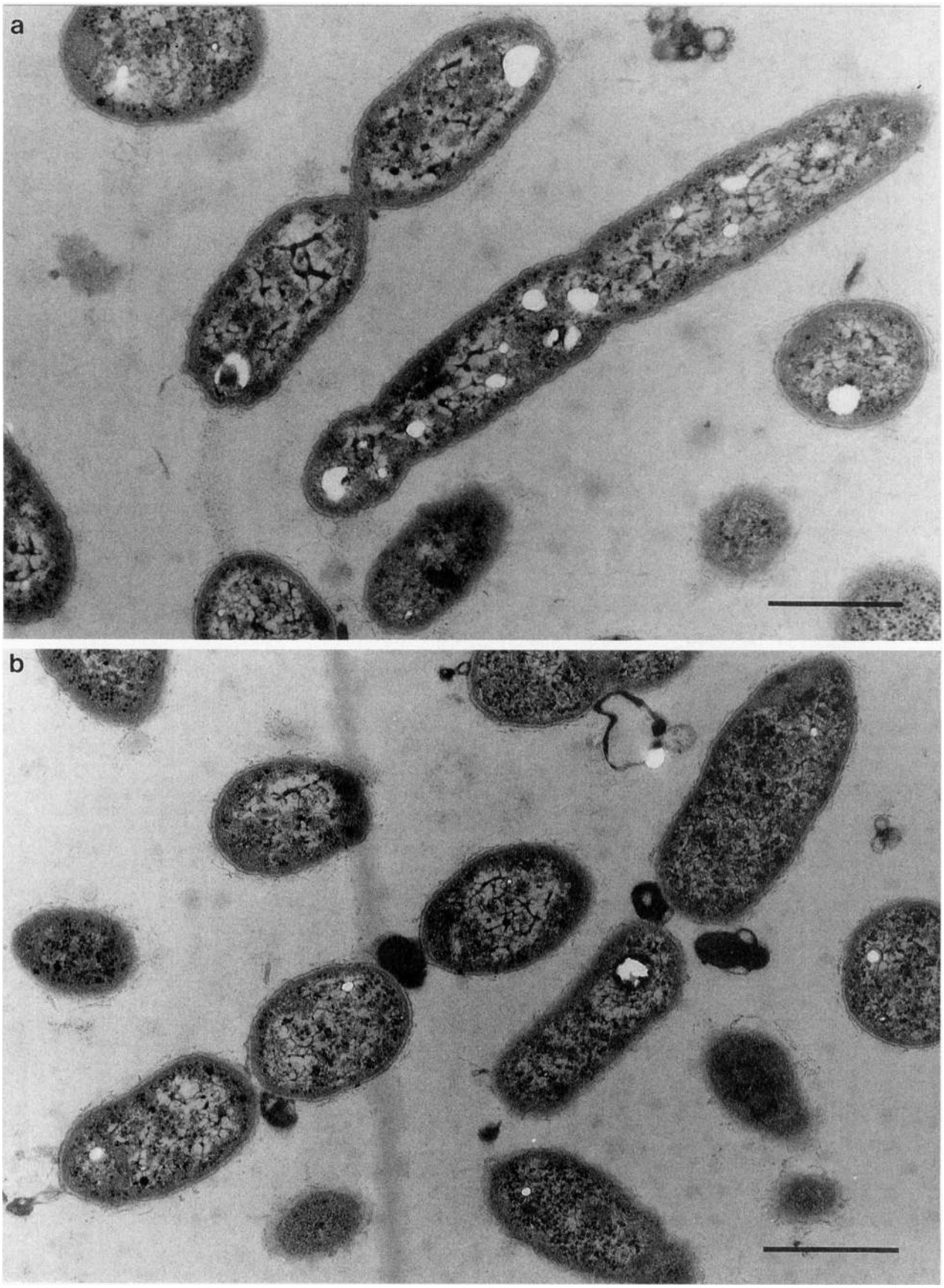


FIG. 2. Thin sections of strains KF1^T and NKF1 and *S. yanoikuyae* DSM 7462^T. (a) Long filamentous cell of strain NKF1 (middle) with a small polarly dividing daughter cell. Bar = 0.5 μm. (b) Newborn NKF1 cells held together by densely staining multilayered material at the point of division. Bar = 0.5 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. yanoikuyae* DSM 7462^T 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.

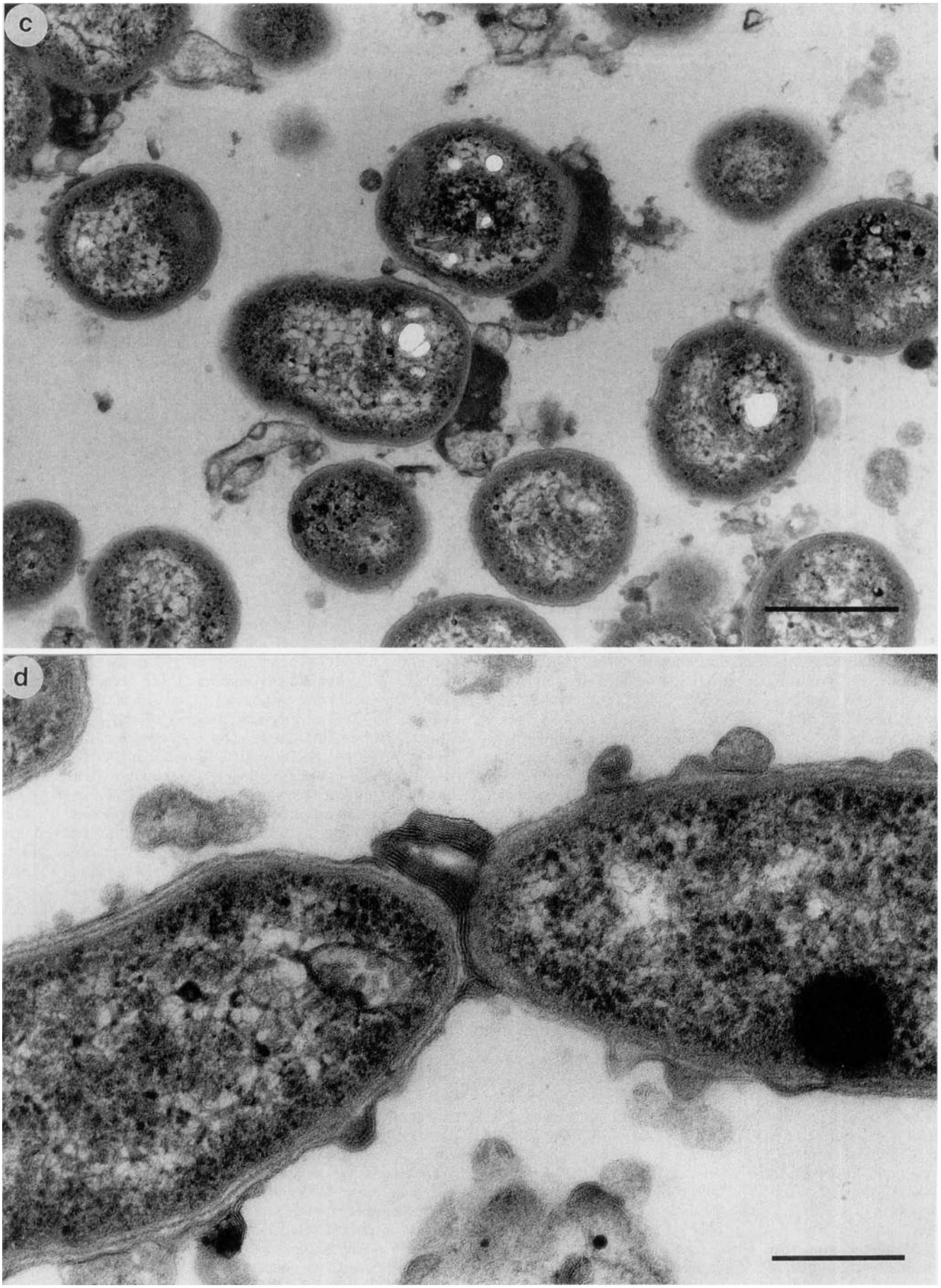


FIG. 2—*Continued.*

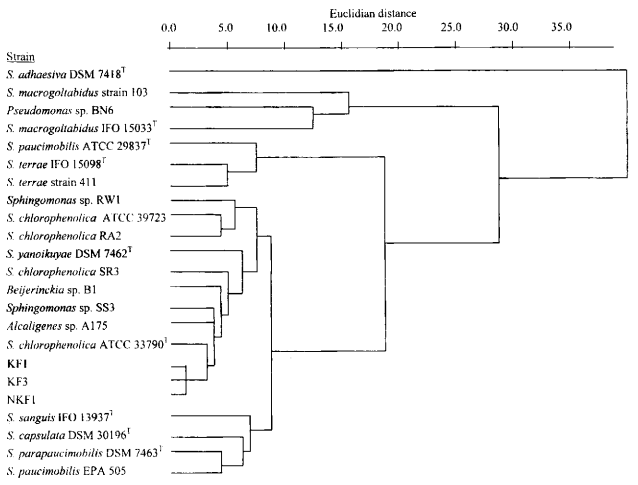


FIG. 3. Euclidean distance dendrogram based on the whole-cell fatty acid compositions of the three polychlorophenol-degrading strains and reference strains.

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 KF1^T, KF3, and NKF1 were found to be identical (Table 4). A comparison of the 16S rDNA sequences of strains KF1^T, KF3, and NKF1 with the sequences of the *Sphingomonas* reference strains (Fig. 5) showed that strains KF1^T, KF3, and NKF1 are the closest relatives of *S. rosa* and *S. capsulata*. Polychlorophenol-degrading strains KF1^T, KF3, and NKF1 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) (56). A comparison of the strain KF1^T, KF3, and NKF1 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).

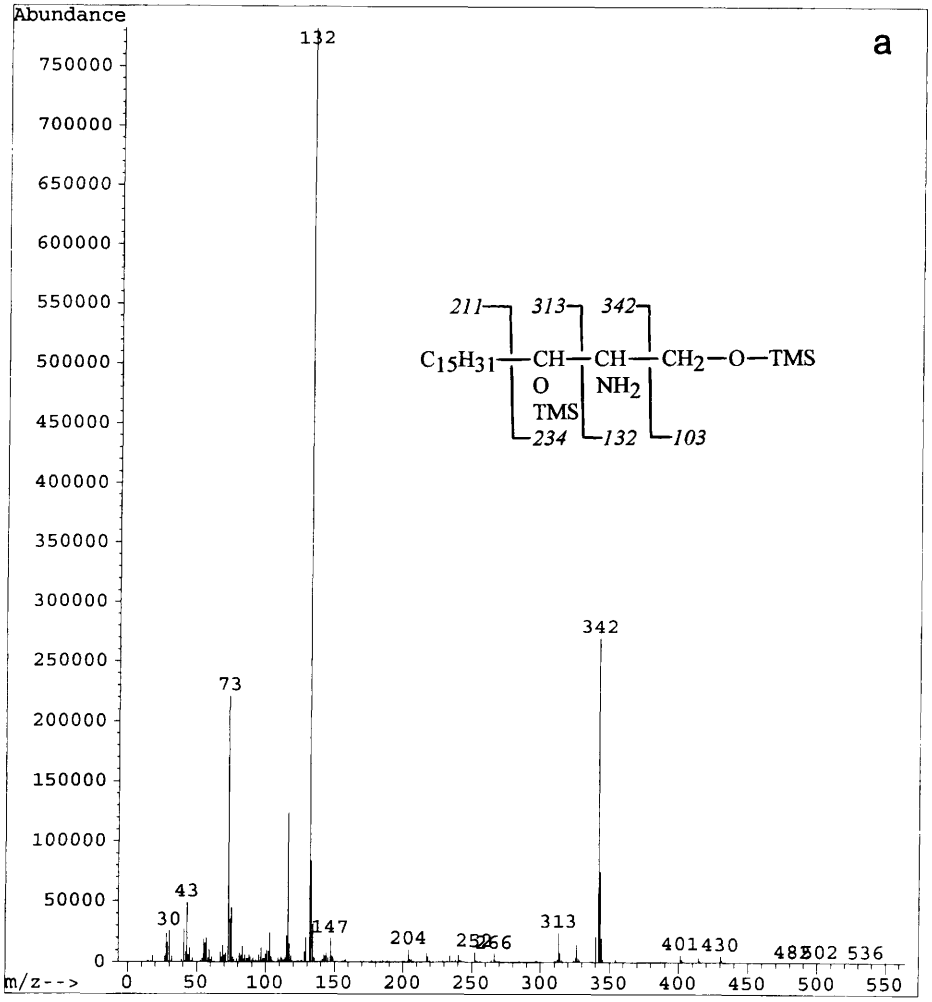
The levels of relatedness between the DNA of strain KF1^T and the DNAs of *S. paucimobilis* ATCC 29837^T, *S. capsulata* DSM 30196^T, *S. yanoikuyae* DSM 7462^T, and *S. chlorophenolica* ATCC 33790^T, ATCC 39723, SR3, and RA2 were less than 40% (Table 5), which confirmed that strains KF1^T, KF3, and NKF1 are members of a separate species. The levels of DNA-DNA relatedness for the chlorophenol-degrading organisms *S. chlorophenolica* ATCC 33790^T, ATCC 39723, SR3, and RA2 ranged from 67 to 97%, but the levels of DNA-DNA relatedness between these strains and strains KF1^T, KF3, and NKF1 were less than 40%. This confirmed our previous description of the four *S. chlorophenolica* strains as members of one species (35).

Metabolic properties. Strains KF1^T, KF3, and NKF1 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 29837^T and *S. capsulata* DSM 30196^T, which were used as reference organisms. The substrates oxidized by strains KF1^T, KF3, and NKF1 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, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, alaninamide, 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 KF1^T, KF3, and NKF1 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 KF1^T, KF3, and NKF1 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).

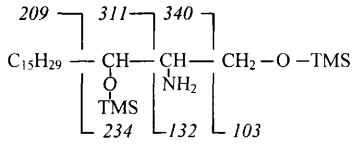
The protein profile dendrogram (Fig. 7) showed that polychlorophenol-degrading strains KF1^T, KF3, and NKF1 were more similar to each other than to reference organisms *S. capsulata* DSM 30196^T, *S. paucimobilis* ATCC 29837^T, and *P. saccharophila* DSM 654^T or to the pentachlorophenol-degrading organisms *S. chlorophenolica* ATCC 33790^T, ATCC 39723, RA2, and SR3.

The results of the cellular lipid component analysis and the polyamine patterns showed that polychlorophenol-degrading strains KF1^T, KF3, and NKF1 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 KF1^T, KF3, and NKF1 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).

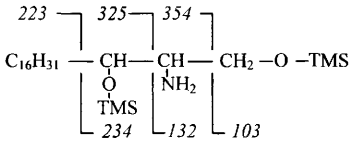
The polychlorophenol-degrading *Sphingomonas* strains formed two distinct clusters on the dendrograms derived from analyses of 16S rDNA sequences, whole-cell proteins, and the ability to oxidize carbon sources. *S. chlorophenolica* ATCC 33790^T, ATCC 39723, RA2, and SR3 fall into one cluster, and *S. subarctica* KF1^T, KF3, and NKF1 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)



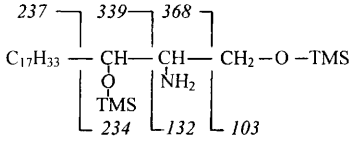
b) d-18:1



c) d-19:1



d) d-20:1



e) d-21:1

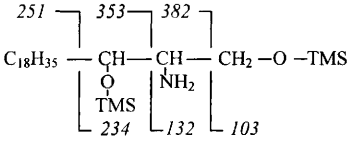


FIG. 4. (a) Mass fragmentogram of the d-18:0 sphingosine peak of strain KF1^T. (b through e) Structural formulas of other trimethylsilyl (TMS) derivatives of sphingosines of strains KF1^T, KF3, and NKF1.

were not located in the same cluster as strains KF1^T, KF3, and NKF1 on the dendrograms constructed in this study (Fig. 3 and 6).

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 KF1^T, 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-

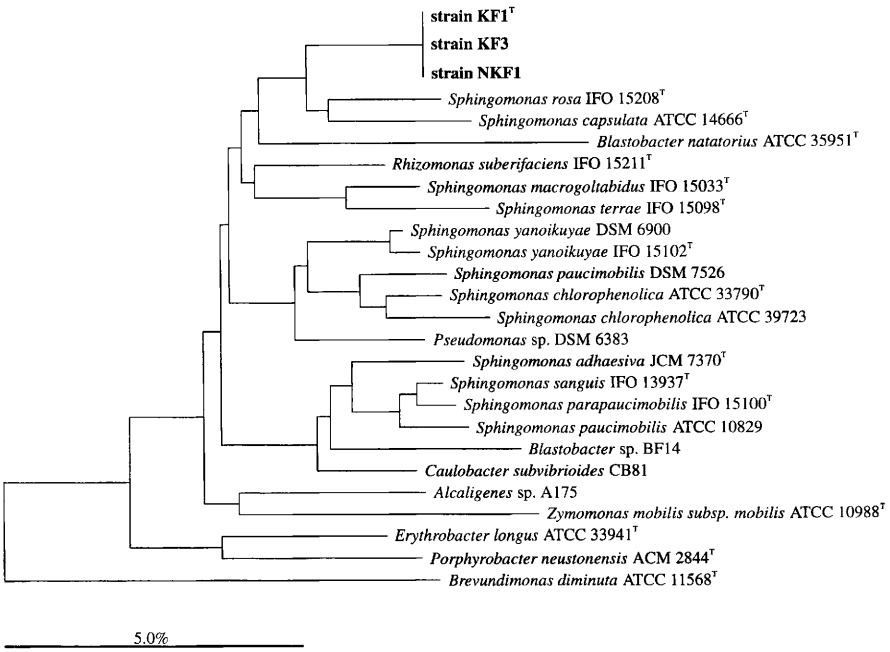


FIG. 5. 16S rDNA-based phylogenetic dendrogram indicating the position of strains KF1^T, KF3, and NKF1 within the radiation of members of the genus *Sphingomonas* and related taxa. Scale bar = 5 nucleotide substitutions per 100 nucleotides. *Beijerinckia* sp. strain B1 has been renamed as *S. yanoikuyae* DSM 6900. *Pseudomonas* sp. BN6 is indicated as *Pseudomonas* sp. DSM 6383, and *S. paucimobilis* EPA 505 is indicated as *Sphingomonas paucimobilis* DSM 7526.

typic and chemotaxonomic properties, including a whole-cell fatty acid analysis, a 16S rDNA analysis, a protein profile analysis, and an analysis of the oxidation of carbon sources by the Biolog system, placed strains KF1^T, KF3, and NKF1 in a taxon that was separate from the previously validly described species of the genus *Sphingomonas*. A comparison of the 16S rDNA sequences of strains KF1^T, KF3, and NKF1 with the sequences reported by Takeuchi et al. (56) resulted in relatively low levels of similarity (94 to 96%). These values demonstrate that isolates KF1^T, KF3, and NKF1 are not related to any of the previously described taxa at the species level, including the recently proposed species *S. pruni*, *S. mali*, *S. rosa*, and *S. asaccharolytica* (56). In addition, polychlorophenol-degrading strains KF1^T, KF3, and NKF1 clearly do not belong to *S. mali*, *S. pruni*, or *S. asaccharolytica* on the basis of the whole-cell fatty acid compositions reported for these species by Takeuchi et al. (56).

So far, two different polyamine patterns have been described for members of the genus *Sphingomonas*. The type strains of *S. paucimobilis*, *S. parapaucimobilis*, *S. pruni*, *S. mali*, and *S. sanguis* have been reported to contain *sym*-homospermidine as the dominant polyamine. *S. yanoikuyae*, *S. capsulata*, *S. rosa*, *S. asaccharolytica*, *S. macrogoltabidus*, and several so-called *Rhizomonas-Sphingomonas*-like strains contain spermidine as the major polyamine (5, 15, 48, 56). *S. adhaesiva* and *S. terrae* have been reported to contain spermidine (56) or *sym*-homospermidine (15, 48). The predominance of spermidine in strains KF1^T, KF3, and NKF1 distinguishes these strains from the taxa containing *sym*-homospermidine, such as *S. paucimobilis*, *S. parapaucimobilis*, *S. pruni*, *S. mali*, and *S. sanguis*. As it has been demonstrated that polyamine patterns are relatively conserved characteristics (5, 15), the different polyamine patterns confirm the conclusion based on 16S rDNA sequence data that strains KF1^T, 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^T, 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^T, KF3, and NKF1 (d-18:0, d-20:1, and d-21:1) were similar to the major dihydrosphin-

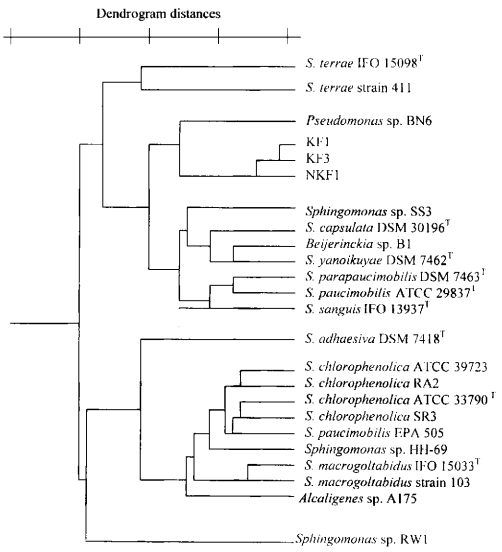


FIG. 6. Dendrogram showing the positions of strains KF1^T, KF3, and NKF1 and strains of related taxa derived from the results of a study of oxidation of 95 different carbon sources as determined by the Biolog GN MicroLog3 method.

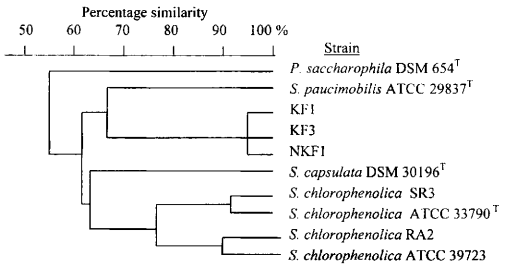


FIG. 7. Protein profile dendrogram derived from whole-cell protein patterns, showing the clustering of strains KF1^T, KF3, and NKF1 and reference strains.

gosines of *S. paucimobilis*, *S. capsulata*, *S. yanoikuyae*, and *S. adhaesiva* (56, 62). The major dihydrosphingosines of *S. sanguis*, *S. macrogoltabidus*, and *S. terrae* have been reported to be d-18:0, d-19:1, and d-20:1 (55). The most abundant sphingosine in the genus *Sphingobacterium* is a branched-chain methylhexadecasphinganine, d-17:0 (7, 63). The glucuronic acid-containing glycosphingolipid of *Z. mobilis* IFO 13756 has been shown to be similar to the glycosphingolipids of *Sphingomonas* strains, differing only in the ceramide moieties (54).

Mainly on the basis of the results of a 16S rDNA sequence comparison, it has been hypothesized previously that the members of the branches represented by *S. yanoikuyae* and *S. capsulata* may belong to a genus different from the genus *Sphingomonas* (48, 57). This hypothesis is supported by the polyamine data. The comparison of the 16S rDNA sequences of the *Sphingomonas* strains and members of related genera belonging to the alpha-4 subgroup of the *Proteobacteria* revealed that members of the genus *Rhizomonas*, two members of the genus *Blastobacter*, and one strain of the genus *Caulobacter*, *C. subvibrioides* CB81, are the members of other genera that are most closely related to the genus *Sphingomonas*. The genus *Rhizomonas* includes only one species, the plant pathogen *R. suberifaciens* (59), which differs from the genus *Sphingomonas* by its lower DNA G+C content (59 mol%). The genera *Blastobacter* and *Caulobacter* are located in the group containing budding bacteria on the basis of morphology, but phylogenetically *Blastobacter* sp. strain BF14, *Blastobacter natatorius* ATCC 35951^T, and *C. subvibrioides* CB81 are closely related to the genus *Sphingomonas* (Fig. 5 and Table 4). Thus, additional chemotaxonomic, physiological, and phylogenetic studies are needed to determine the taxonomic positions of bacteria belonging to the alpha-4 subgroup of the *Proteobacteria.*

The ultrastructure revealed by electron micrographs of cells of strains KF1^T, KF3, and NKF1 was very similar to the ultrastructure of *S. chlorophenolica* (35). *S. yanoikuyae* DSM 7462^T was included in this study as a reference organism, because it was phylogenetically closely related to *S. chlorophenolica* (23). So far, *S. chlorophenolica* is the only *Sphingomonas* species whose ultrastructure has been described thoroughly (35). The phylogenetic tree for the *Sphingomonas* strains revealed relatedness to *Blastobacter natatorius* ATCC 35951^T (50) and *Z. mobilis* subsp. *mobilis* ATCC 10988^T (9, 52), whose ultrastructures have been described. Interestingly, these organisms have ultrastructural features that are shared by strains KF1^T, KF3, and NKF1, *S. chlorophenolica*, and *S. yanoikuyae* DSM 7462^T, as determined in this study. *Sphingomonas* cells typically form concentrically arranged layered membranous blebs that extrude from the outer membrane and accumulate especially at the site of cell division, as shown in this study (Fig. 2) and by Nohynek et al. (35). The plasma membrane and peptidoglycan grow symmetrically inward into the cytoplasm at the site of cell division, leaving the outer membrane behind at the cell surface, and the outer membrane continues to grow and to extrude blebs into the extracellular space. A phenomenon resembling this process was first described for *Z. mobilis* (3). Also, cell division seems to occur more often asymmetrically than symmetrically (Fig. 2a and b) (35), a phenomenon which has been observed previously with *Blastobacter* cells (50). Similar cell division has been described previously for another sphingolipid-containing taxon, *Sphingobacterium multivorum* (18), which is phylogenetically clearly separate from the genus *Sphingomonas*. Both strains KF1^T, KF3, and NKF1 and *S. chlorophenolica* possess fimbriae, but fimbriae have not been found yet in related taxa. Old cultures of strains KF1^T, KF3, and NKF1 became slimy, especially when they were stored at temperatures below room temperature. Several *Sphingomonas* species, as well as *Zymomonas* species, have been intensively studied for industrial purposes because of their ability to produce extracellular polysaccharides (1, 27, 30, 37).

We propose that strains KF1^T, KF3, and NKF1 should be placed in a new species in the genus *Sphingomonas*, *S. subarctica*. Interestingly, our 16S rDNA data (Fig. 5 and Table 4) show that several other xenobiotic compound degraders (e.g., 1,4-dichlorobenzene-degrading *Alcaligenes* sp. strain A175 [47] and substituted naphthalenesulfonate-degrading *Pseudomonas* sp. strain BN6 [36]) should be reclassified as members of *Sphingomonas* species. The polyaromatic hydrocarbon-utilizing organism *Beijerinckia* sp. strain B1 (12, 28), which was recently reclassified as *S. yanoikuyae* B1 (26), was used in this study as a reference strain. The taxonomic location of this strain as a member of *S. yanoikuyae* determined in this study was based in part on criteria similar to the criteria used in the study of Khan et al. (26), such as 16S rDNA sequence and whole-cell fatty acid composition. The interest in the genus *Sphingomonas* is increasing, because there have been several recent reports of *Sphingomonas* strains that degrade xenobiotic compounds, including polycyclic aromatic hydrocarbons (24, 32, 53), 2,4-dichlorophenoxyacetic acid (22), dibenzo-*p*-dioxin and dibenzofuran (17, 31, 60), lindane (33), diphenyl ether (46), and synthetic polymers, such as polyethylene glycols (25, 55).

Description of *Sphingomonas subarctica* sp. nov. *S. subarctica* (sub.arc'ti.ca. M.L. adj. *subarcticus*, 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. Mesophilic and aerobic. Esculin and *p*-nitrophenyl-β-D-galactopyranoside positive. Utilizes 2,4,6-tri- and 2,3,4,6-tetrachlorophenols and oxidizes 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, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, alaninamide, 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. Assimilates glucose, arabinose, *N*-acetylglucosamine, maltose, malate, and citrate. The main dihydrosphingosines are d-18:0, d-18:1, d-19:1, d-20:1, and d-21:1. The main cellular fatty acids are octadecenoic, 2-hydroxymyristic, *cis*-9-hexadecenoic, and hexadecanoic acids. The main respiratory quinone is ubiquinone 10, the major polyamine is spermidine, and the G+C content of the DNA is 66 ± 1 mol%. *S. subarctica* differs

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*-acetylglucosamine, maltose, and citrate in API 20NE tests. *S. subarctica* KF1^T (= HAMBI 2110^T), KF3 (= HAMBI 2111), and NKF1 (= HAMBI 2112) degrade 2,4,6-tri- and 2,3,4,6-tetrachlorophenols but not pentachlorophenol. The type strain is strain KF1 (= HAMBI 2110).

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REFERENCES

1. **Ashtaputre, A. A., and A. K. Shah.** 1995. Studies on a viscous, gel-forming exopolysaccharide from *Sphingomonas paucimobilis* GS1. Appl. Environ. Microbiol. **61:**1159–1162.
2. **Asturias, J. A., E. Moore, M. M. Yakimov, S. Klatte, and K. N. Timmis.** 1994. Reclassification of the polychlorinated biphenyl-degraders *Acinetobacter* sp. strain P6 and *Corynebacterium* sp. strain MB1 as *Rhodococcus globerulus*. Syst. Appl. Microbiol. **17:**226–231.
3. **Beveridge, T., and R. Harris.** 1984. Septation in *Zymomonas mobilis*. Can. J. Microbiol. **30:**1283–1289.
4. **Briglia, M., R. Eggen, D. van Elsas, and W. de Vos.** 1994. Phylogenetic evidence for the transfer of the pentachlorophenol-mineralizing *Rhodococcus chlorophenolicus* strain PCP-1 to the genus *Mycobacterium*. Int J. Syst. Bacteriol. **44:**494–498.
5. **Busse, J., and G. Auling.** 1988. Polyamine patterns as a chemotaxonomic marker within the *Proteobacteria*. Syst. Appl. Microbiol. **11:**1–8.
6. **Cashion, P., M. A. Holder-Franklin, J. McCully, and M. A. Holder-Franklin.** 1977. A rapid method for the base ratio determination of bacterial DNA. Anal. Biochem. **81:**461–466.
7. **Dees, S. B., G. M. Carlone, D. Hollis, and W. Moss.** 1985. Chemical and phenotypic characteristics of *Flavobacterium thalpophilum* compared with those of other *Flavobacterium* and *Sphingobacterium* species. Int. J. Syst. Bacteriol. **35:**16–22.
8. **De Ley, J., H. Cattoir, and A. Reynaerts.** 1970. The quantitative measurement of DNA hybridization from renaturation rates. Eur. J. Biochem. **12:** 133–142.
9. **Doelle, H. W., H.-J. Preusser, and H. Rostek.** 1982. Electron microscopic investigations of *Zymomonas mobilis* cells grown in low and high glucose concentrations. Eur. J. Appl. Microbiol. Biotechnol. **16:**136–141.
10. **Edgehill, R. U., and R. K. Finn.** 1982. Isolation, characterization and growth kinetics of bacteria metabolizing pentachlorophenol. Eur. J. Appl. Microbiol. Biotechnol. **16:**179–184.
11. **Fuerst, J. A., J. A. Hawkins, A. Holmes, L. I. Sly, C. J. Moore, and E. Stackebrandt.** 1993. *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from freshwater. Int. J. Syst. Bacteriol. **43:**125–134.
12. **Gibson, D. T., R. L. Roberts, M. C. Wells, and V. M. Kobal.** 1973. Oxidation of biphenyl by a *Beijerinckia* sp. Biochem. Biophys. Res. Commun. **50:**211–219.
13. **Häggblom, M., L. Nohynek, N. Palleroni, K. Kronqvist, E.-L. Nurmiaho-Lassila, M. Salkinoja-Salonen, S. Klatte, and R. Kroppenstedt.** 1994. Transfer of polychlorophenol-degrading *Rhodococcus chlorophenolicus* (Apajalahti et al. 1986) to the genus *Mycobacterium* as *Mycobacterium chlorophenolicum* comb. nov. Int. J. Syst. Bacteriol. **44:**485–493.
14. **Häggblom, M. M., and R. J. Valo.** 1995. Bioremediation of chlorophenol wastes, p. 389–434. *In* L. Young and C. Cerniglia (ed.), Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss, Inc., New York.
15. **Hamana, K., and S. Matsuzaki.** 1993. Polyamine distribution patterns serve a phenotypic marker in the chemotaxonomy of the *Proteobacteria*. Can. J. Microbiol. **39:**304–310.
16. **Hantula, J., A. Kurki, P. Vuoriranta, and D. Bamford.** 1991. Rapid classification of bacterial strains by SDS-polyacrylamide gel electrophoresis: population dynamics of the dominant dispersed phase bacteria of activated sludge. Appl. Microbiol. Biotechnol. **34:**551–555.
17. **Harms, H., H. Wilkes, R.-M. Wittich, and P. Fortnagel.** 1995. Metabolism of hydroxybenzofurans, methoxydibenzofurans, acetoxydibenzofurans, and nitrodibenzofurans by *Sphingomonas* sp. strain HH69. Appl. Environ. Microbiol. **61:**2499–2505.
18. **Hayward, A. C., and L. I. Sly.** 1984. Characterization of a dextranolytic biotype of *Flavobacterium multivorum* from soil. J. Appl. Bacteriol. **57:**505–516.
19. **Huss, V. A. R., H. Festl, and K. H. Schleifer.** 1983. Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst. Appl. Microbiol. **4:**184–192.
20. **Janke, K.-D.** 1992. BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD SYSTEM 2600 spectrophotometer on a PC/XT/AT type personal computer. J. Microbiol. Methods **15:**61–73.
21. **Jukes, T. H., and C. R. Cantor.** 1969. Evolution of protein molecules, p. 21–132. *In* H. N. Munro (ed.), Mammalian protein metabolism. Academic Press, New York.
22. **Ka, J. O., W. E. Holben, and J. M. Tiedje.** 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.
23. **Karlson, U., F. Rojo, J. D. van Elsas, and E. Moore.** 1995. Genetic and serological evidence for recognition of four pentachlorophenol-degrading bacterial strains as species of the genus *Sphingomonas*. Syst. Appl. Microbiol. **18:**539–548.
24. **Kästner, M., M. Breuer-Jammali, and B. Mahro.** 1994. Enumeration and characterization of the soil microflora from hydrocarbon-contaminated soil sites able to mineralize polycyclic aromatic hydrocarbons (PAH). Appl. Microbiol. Biotechnol. **41:**267–273.
25. **Kawai, F., and H. Yamanaka.** 1986. Biodegradation of polyethylene glycol by symbiotic mixed culture (obligate mutualism). Arch. Microbiol. **146:**125–129.
26. **Khan, A. A., R.-F. Wang, W.-W. Cao, W. Franklin, and C. E. Cerniglia.** 1996. Reclassification of a polycyclic aromatic hydrocarbon-metabolizing bacterium, *Beijerinckia* sp. strain B1, as *Sphingomonas yanoikuyae* by fatty acid analysis, protein pattern analysis, DNA-DNA hybridization, and 16S ribosomal DNA sequencing. Int. J. Syst. Bacteriol. **46:**466–469.
27. **Lobas, D., M. Nimtz, V. Wray, A. Schumpe, C. Proppe, and W.-D. Deckwer.** 1994. Structure and physical properties of the extracellular polysaccharide PS-P4 produced by *Sphingomonas paucimobilis* P4 (DSM 6418). Carbohydr. Res. **251:**303–313.
28. **Mahaffey, W. R., D. T. Gibson, and C. E. Cerniglia.** 1988. Bacterial oxidation of chemical carcinogens: formation of polycyclic aromatic acids from benz [*a*]anthracene. Appl. Environ. Microbiol. **54:**2415–2423.
29. **Maidak, B. L., N. Larsen, M. J. McCaughey, R. Overbeck, G. J. Olsen, K. Fogel, J. Blandy, and C. R. Woese.** 1994. The Ribosomal Database Project. Nucleic Acids Res. **22:**3483–3487.
30. **Martins, L. O., and I. Sá-Correia.** 1993. Temperature profiles of gellan gum synthesis and activities of biosynthetic enzymes. Biotechnol. Appl. Biochem. **20:**385–395.
31. **Moore, E. R. B., R.-M. Wittich, P. Fortnagel, and K. N. Timmis.** 1993. 16S ribosomal RNA gene sequence characterization and phylogenetic analysis of a dibenzo-*p*-dioxin-degrading isolate within the new genus *Sphingomonas*. Lett. Appl. Microbiol. **17:**115–118.
32. **Muller, J. G., P. J. Chapman, B. O. Blattman, and P. H. Pritchard.** 1990. Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. Appl. Environ. Microbiol. **56:**1079–1086.
33. **Nishiyama, M., K. Senoo, and S. Matsumoto.** 1993. Establishment of γ-1,2,3,4,5,6-hexachlorocyclohexane-assimilating bacterium, *Sphingomonas paucimobilis* strain SS86, in soil. Soil Biol. Biochem. **25:**769–774.
34. **Nohynek, L., M. Häggblom, N. Palleroni, K. Kronqvist, E.-L. Nurmiaho-Lassila, and M. Salkinoja-Salonen.** 1993. Characterization of a *Mycobacterium fortuitum* strain capable of degrading polychlorinated phenolic compounds. Syst. Appl. Microbiol. **16:**126–134.
35. **Nohynek, L., E. Suhonen, E.-L. Nurmiaho-Lassila, J. Hantula, and M. Salkinoja-Salonen.** 1995. Description of four pentachlorophenol-degrading bacterial strains as *Sphingomonas chlorophenolica* sp. nov. Syst. Appl. Microbiol. **18:**527–538.
36. **Nörtemann, B., A. E. Kuhm, H.-J. Knackmuss, and A. Stolz.** 1994. Conversion of substituted naphthalenesulfonates by *Pseudomonas* sp. BN6. Arch. Microbiol. **161:**320–327.
37. **Pollock, T. J.** 1993. Gellan-related polysaccharides and the genus *Sphingomonas*. J. Gen. Microbiol. **139:**1939–1945.
38. **Puhakka, J. A., R. P. Herwig, P. M. Koro, G. V. Wolfe, and J. F. Ferguson.** 1995. Biodegradation of chlorophenols by mixed and pure cultures from a fluidized-bed reactor. Appl. Microbiol. Biotechnol. **42:**951–957.
39. **Radehaus, P. M., and S. K. Schmidt.** 1992. Characterization of a novel *Pseudomonas* sp. that mineralizes high concentrations of pentachlorophenol.

Appl. Environ. Microbiol. **58:**2879–2885.

40. **Rainey, F. A., M. Dorsch, H. W. Morgan, and E. Stackebrandt.** 1992. 16S rDNA analysis of *Spirochaeta thermophila*: its phylogenetic position and implications for the systematics of the order *Spirochaetales*. Syst. Appl. Microbiol. **16:**224–226.
41. **Rainey, F. A., and E. Stackebrandt.** 1993. 16S rDNA analysis reveals phylogenetic diversity among the polysaccharolytic clostridia. FEMS Microbiol. Lett. **113:**125–128.
42. **Resnick, S., and P. Chapman.** 1994. Physiological properties and substrate specificity of a pentachlorophenol-degrading *Pseudomonas* species. Biodegradation **5:**47–54.
43. **Saber, D., and R. Crawford.** 1985. Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. Appl. Environ. Microbiol. **50:**1512–1518.
44. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
45. **Scherer, P., and H. Kneifel.** 1983. Distribution of polyamines in methanogenic bacteria. J. Bacteriol. **154:**1315–1322.
46. **Schmidt, S., R.-M. Wittich, D. Erdmann, H. Wilkes, W. Francke, and P. Fortnagel.** 1992. Biodegradation of diphenyl ether and its monohalogenated derivatives by *Sphingomonas* sp. strain SS3. Appl. Environ. Microbiol. **58:** 2744–2750.
47. **Schraa, G., M. L. Boone, M. S. M. Jetten, A. R. W. van Neerven, P. J. Colberg, and A. J. B. Zehnder.** 1986. Degradation of 1,4-dichlorobenzene by *Alcaligenes* sp. strain A175. Appl. Environ. Microbiol. **52:**1374–1381.
48. **Segers, P., M. Vancanneyt, B. Pot, U. Torck, B. Hoste, D. Dewettinck, E. Falsen, K. Kersters, and P. de Vos.** 1994. Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Büsing, Doll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. Int. J. Syst. Bacteriol. **44:**499–510.
49. **Shiba, T., and U. Simidu.** 1982. *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll *a*. Int. J. Syst. Bacteriol. **32:**211–217.
50. **Sly, L. I.** 1985. Emendation of the genus *Blastobacter* Zavarzin 1961 and description of *Blastobacter natatorius* sp. nov. Int. J. Syst. Bacteriol. **35:**40–45.
51. **Sneath, P. H. A., and R. R. Sokal.** 1973. Numerical taxonomy: the principles and practice of numerical classification, p. 394–400. W. H. Freeman, San Francisco.
52. **Stevnsborg, N., H. G. Lawford, N. Martin, and T. Beveridge.** 1986. Effect of growth temperature on the morphology and performance of *Zymomonas mobilis* ATCC 29191 in batch and continuous culture. Appl. Microbiol. Biotechnol. **25:**116–123.
53. **Stringfellow, W., and M. Aitken.** 1994. Comparative physiology of phenanthrene degradation by two dissimilar pseudomonads isolated from a creosote-contaminated soil. Can. J. Microbiol. **40:**432–438.
54. **Tahara, Y., and M. Kawazu.** 1994. Isolation of glucuronic acid-containing glycosphingolipids from *Zymomonas mobilis*. Biosci. Biotechnol. Biochem. **58:**586–587.
55. **Takeuchi, M., F. Kawai, Y. Shimada, and A. Yokota.** 1993. Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. Syst. Appl. Microbiol. **16:**227–238.
56. **Takeuchi, M., T. Sakane, M. Yanagi, K. Yamasato, K. Hamana, and A. Yokota.** 1995. Taxonomic study of bacteria isolated from plants: proposal of *Sphingomonas rosa* sp. nov., *Sphingomonas pruni* sp. nov., *Sphingomonas asaccharolytica* sp. nov. and *Sphingomonas mali* sp. nov. Int. J. Syst. Bacteriol. **45:**334–341.
57. **Takeuchi, M., H. Sawada, H. Oyaizu, and A. Yokota.** 1994. Phylogenetic evidence for *Sphingomonas* and *Rhizomonas* as nonphotosynthetic members of the alpha-4 subclass of the *Proteobacteria*. Int. J. Syst. Bacteriol. **44:**308–314.
58. **Väisänen, O., E.-L. Nurmiaho-Lassila, S. Marmo, and M. Salkinoja-Salonen.** 1994. Structure and composition of biological slimes of paper and board machines. Appl. Environ. Microbiol. **60:**641–653.
59. **van Bruggen, A. H. C., K. N. Jochimsen, and P. R. Brown.** 1990. *Rhizomonas suberifaciens* gen. nov., sp. nov., the causal agent of corky root of lettuce. Int. J. Syst. Bacteriol. **40:**175–188.
60. **Wittich, R.-M., H. Wilkes, V. Sinnwell, W. Francke, and P. Fortnagel.** 1992. Metabolism of dibenzo-*p*-dioxin by *Sphingomonas* sp. strain RW1. App. Environ. Microbiol. **58:**1005–1010.
61. **Yabuuchi, E., T. Kaneko, I. Yano, C. W. Moss, and N. Miyoshi.** 1983. *Sphingobacterium* gen. nov., *Sphingobacterium spiritivorum* comb. nov., *Sphingobacterium multivorum* comb. nov., *Sphingobacterium mizutae* sp. nov., and *Flavobacterium indologenes* sp. nov.: glucose-nonfermenting gram-negative rods in CDC groups IIK-2 and IIb. Int. J. Syst. Bacteriol. **33:**580–598.
62. **Yabuuchi, E., I. Yano, H. Oyaizu, Y. Hashimoto, T. Ezaki, and H. Yamamoto.** 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.
63. **Yano, I., I. Tomiyasu, and E. Yabuuchi.** 1982. Long chain base composition of strains of three species of *Sphingobacterium* gen. nov. FEMS Microbiol. Lett. **15:**303–307.