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Taxonomic Study of Aromatic-Degrading Bacteria from Deep-Terrestrial-Subsurface Sediments and Description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov.

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Phylogenetic analyses of 16S rRNA gene sequences by distance matrix and parsimony methods indicated that six strains of bacteria isolated from deep saturated Atlantic coastal plain sediments were closely related to the genus *Sphingomonas*. Five of the strains clustered with, but were distinct from, *Sphingomonas capsulata*, whereas the sixth strain was most closely related to *Blastobacter natatorius*. The five strains that clustered with *S. capsulata*, all of which could degrade aromatic compounds, were gram-negative, non-spore-forming, non-motile, rod-shaped organisms that produced small, yellow colonies on complex media. Their G+C contents ranged from 60.0 to 65.4 mol%, and the predominant isoprenoid quinone was ubiquinone Q-10. All of the strains were aerobic and catalase positive. Indole, urease, and arginine dihydrolase were not produced. Gelatin was not liquified, and glucose was not fermented. Sphingolipids were present in all strains; 2OH14:0 was the major hydroxy fatty acid, and 18:1 was a major constituent of cellular lipids. Acid was produced oxidatively from pentoses, hexoses, and disaccharides, but not from polyalcohols and indole. All of these characteristics indicate that the five aromatic-degrading strains should be placed in the genus *Sphingomonas* as currently defined. Phylogenetic analysis of 16S rRNA gene sequences, DNA-DNA reassociation values, BOX-PCR genomic fingerprinting, differences in cellular lipid composition, and differences in physiological traits all indicated that the five strains represent three previously undescribed *Sphingomonas* species. Therefore, we propose the following new species: *Sphingomonas aromaticivorans* (type strain, SMCC F199), *Sphingomonas subterranea* (type strain, SMCC B0478), and *Sphingomonas stygia* (type strain, SMCC B0712).

Bacteria inhabit deep subsurface rocks and sediments and can influence the geochemistry of their surrounding environment (9, 19, 43, 45, 51). Preliminary physiological studies of isolates obtained from deep Atlantic coastal plain sediments in the United States suggested that the subsurface bacterial populations in those sediments are metabolically diverse (2, 4, 23). However, only limited efforts have been made to examine the phylogenetic traits of these isolates or to determine their taxonomic status (7, 46).

Because of the potential for in situ biodegradation of petroleum hydrocarbons in contaminated groundwater (i.e., bioremediation), there is considerable interest in bacteria that can degrade these compounds. One such organism, strain F199^T (T = type strain), was among the isolates obtained from deep Atlantic coastal plain sediments mentioned above and was found to degrade a broad range of aromatic compounds, including xylene, toluene, and naphthalene (18). The ability to degrade these compounds is encoded on a 180-kb plasmid in F199^T (52), which may have evolved because sedimentary organic material is the principal source of energy in this organism's environment (18). Strain F199^T and several other subsurface bacterial isolates that can degrade various aromatic compounds were subsequently shown to be members of the genus *Sphingomonas* (16).

The genus *Sphingomonas* was described by Yabuuchi et al. (64) and later was emended by Takeuchi et al. (55). Organisms in this genus are gram-negative, non-spore-forming rods that have a single polar flagellum when they are motile. They are yellow and obligately aerobic and produce catalase. Acid is produced oxidatively from pentoses, hexoses, and disaccharides, but not from polyalcohols and inulin. The major respiratory quinone is ubiquinone Q-10, and the major fatty acids of the cellular lipid are 18:1 and 2OH14:0. The cellular lipid contains sphingoglycolipid. The G+C contents of the genomic DNAs range from 61.6 to 67.8 mol%.

The genus *Sphingomonas* appears to be ubiquitous in soil, water, and sediments. *Sphingomonas* strains isolated from these environments have broad catabolic capabilities and, therefore, have high potential for bioremediation and waste treatment. Among the contaminants that can be degraded by various *Sphingomonas* species are dibenzo-*p*-dioxin and dibenzofuran (63); hexachlorocyclohexane (25); chlorinated biphenyls (54); pentachlorophenol (29); halogenated diphenyl ethers (48); naphthalenesulfonic acids (33); toluene, naphthalene, and xylene (18); and polyaromatic hydrocarbons (31, 42). Of the aromatic-degrading *Sphingomonas* strains described to date, *Sphingomonas yanoikuyae* B1 has been studied most extensively at the biochemical and molecular levels. A recent genetic homology study (32) showed that the genes for biphenyl and *m*-xylene degradation in this strain were similar to the genes in

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another surface soil isolate (*S. yanoikuyae* Q1) and five deep-subsurface strains (F199^T, B0522, B0695, B0478^T, and B0712^T). However, the degradative genes in the surface strains were located on the chromosome, whereas the degradative genes in the subsurface strains were located on plasmids.

In this study, we describe the morphological, biochemical, and phylogenetic characteristics of aromatic-degrading subsurface *Sphingomonas* strains isolated from deep Atlantic coastal plain sediments. We also propose that five of the subsurface strains be assigned to three new species of *Sphingomonas*, *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov.

MATERIALS AND METHODS

Sources of bacterial strains and maintenance of cultures. *Sphingomonas capsulata* ATCC 14666, *Sphingomonas paucimobilis* ATCC 29837, and *S. yanoikuyae* ATCC 51230 were obtained from the American Type Culture Collection. *S. yanoikuyae* B1 and Q1 (see references 31 and 32 were obtained from Gerben J. Zylstra (Rutgers University, New Brunswick, N.J.). The subsurface bacterial strains used in this study (B0477, B0478^T, B0522, B0695, B0712^T, and F199^T), which were isolated from deep saturated Atlantic coastal plain sediments (2, 17), were obtained from the Department of Energy (DOE) Subsurface Microbial Culture Collection (SMCC) at Florida State University (3). All strains were cultured on 1% PTYG medium (5) and were maintained as described previously (16). For G+C content analyses, bacteria were grown in half-strength Luria broth at 30°C with rotary shaking; 100-ml cultures were inoculated from single colonies (grown on 0.5× Luria broth agar), incubated overnight, and transferred to 1 liter of fresh medium. The cultures were incubated at 30°C for 2 days and then harvested by centrifugation.

Microscopy. Gram staining was performed by the Hucker method (12). Capsule staining was performed by the India ink method (12).

Physiological characterization. Selected physiological traits of *S. capsulata*, *S. paucimobilis*, and the subsurface strains were determined with API NFT (nine metabolic capabilities and aerobic growth on 12 carbon sources), API 50CH (utilization of and oxidative acid production from 49 carbohydrates and related compounds), and API ZYM (19 enzymatic activities) test kits (bioMérieux-Vitek, Inc., Hazelwood, Mo.). All API tests were performed in accordance with the manufacturer's directions. Fermentative acid production and oxidative acid production from 25 carbohydrates were tested by growth in OF basal medium (Difco Laboratories, Detroit, Mich.) (soft-agar stabs with and without sterile mineral oil overlay, respectively) supplemented with 0.5 to 1% carbohydrate. The OF medium tubes were incubated at 30°C for 10 days, during which reactions were recorded every 2 days.

Phylogenetic analysis. Genomic DNAs were isolated from *S. paucimobilis* ATCC 29837 and the subsurface strains by a standard chloroform-isoamyl alcohol extraction procedure (26). Twenty nanograms of DNA was then used as a template for PCR amplification (47) of an approximately 1,500-base segment of the 16S rRNA gene (i.e., nearly the entire gene). The PCR amplification primers used were primers fD1 (AGAGTTTGATCCTGGCTCAG) and rP2 (ACGGCTACCTTGTTACGACTT) (62).

The PCR amplification products were sequenced with an Applied Biosystems model 373A DNA sequencer by using the *Taq* DyeDeoxy terminator cycle sequencing method (1, 39). The following primers were used to sequence all six strains: primer C (ACGGGCGGTGTGTAC), corresponding to positions 1406 to 1392 in the 16S ribosomal DNA (rDNA) nucleotide sequence of *Escherichia coli* (8); primer H (ACACGAGCTGACGACAGCCA; *E. coli* positions 1075 to 1056); primer G (CCAGGGTATCTAATCCTGTT; *E. coli* positions 800 to 781); primer A (GTATTACCGCGG[C/G]TGCTG; *E. coli* positions 536 to 519); and primer P (CTGCTGCCTCCCGTAGGAG; *E. coli* positions 357 to 339). One or more the following complementary primers were used as needed (to resolve ambiguous bases or to obtain additional information) for sequencing of some strains: primers F$_2$C (AGAGTTTGATC[A/C]TGGCTC; positions 8 to 25), PC (CTACGGGAGGCAGCAG; positions 342 to 357), AC (CAGCCGCGGTAATAC; positions 522 to 536), GC (AACAGGATTAGATACCCTGG; positions 781 to 800), and HC (TGGCTGTCGTCAGCTCGTGT; positions 1056 to 1075). Primers A and C were described by Lane et al. (34), and primers P and PC were described (as primers 339-357 and 357-342, respectively) by Weisburg et al. (62). The remaining primers were developed by R. H. Reeves and J. Y. Reeves at Florida State University. The resulting sequences were assembled to produce 1,304-base contiguous rDNA sequences corresponding to *E. coli* positions 30 to 1375. Between 70 and 85% of the contiguous sequences for each strain could be read from more than one primer during assembly.

A previous analysis of the 16S rRNA gene sequences of strains F199^T and B0695 (16) indicated that these organisms fall in the alpha subclass of the *Proteobacteria* and are probably members of the genus *Sphingomonas* (55, 64). To more precisely determine the phylogenetic position of these strains and the other subsurface strains within the genus *Sphingomonas* in the present study, their 16S rDNA sequences were hand aligned with the corresponding sequences for 54 selected strains of eubacteria (Table 1). Included in this alignment were (i) all of the *Sphingomonas* sequences (including more than 150 bases) currently available from GenBank, the European Molecular Biology Laboratory (EMBL) database, and the Ribosomal Database Project (RDP), version 5 (36); (ii) sequences of 11 species belonging to the alpha subclass of the *Proteobacteria* and closely related to the genus *Sphingomonas*; and (iii) an *Arthrobacter globiformis* sequence (which was used as an outgroup). The aligned sequences were then analyzed by parsimony and distance matrix methods (see below). This analysis was limited to three regions (corresponding to *E. coli* positions 227 to 501, 720 to 894, and 1180 to 1375; a total of approximately 622 bases) for which sequences were available for all 60 strains in the alignment set (including the subsurface strains).

Based on the phylogenetic trees produced by the analyses described above (data not shown), 41 sequences were selected for an analysis of a more complete portion of the 16S rRNA gene. This alignment set included the sequences of the subsurface strains, 24 representative *Sphingomonas* strains (including the strains that were most closely related to the subsurface strains according to the analysis described above), 10 representative species belonging to the alpha subclass of the *Proteobacteria*, and *A. globiformis* (used as the outgroup). The aligned sequences were analyzed with parsimony and distance matrix methods (see below). This analysis included a region corresponding to *E. coli* positions 30 to 1375, from which a few small segments of sequence were excluded because the alignment was ambiguous in those regions. (A total of 1,292 bases were retained for analysis.)

Maximum-parsimony analysis was performed with the program Phylogenetic Analysis Using Parsimony, Macintosh version 3.1.1 (PAUP) (53). Only the phylogenetically informative sites were considered. A heuristic search was carried out first (by using the standard program defaults), after which a bootstrap analysis was used to evaluate the branch points of the resulting phylogenetic trees. Consensus phylogenetic trees were produced by bootstrapping at the greater-than-50% confidence limit, with 100 replications (13).

The distance matrix analysis was carried out by using the PHYLIP package of computer programs (14). Distances were calculated by the method of Jukes and Cantor (28), after which phylogenies were estimated with the FITCH option (which makes use of the Fitch-Margoliash criterion [15] and some related least-squares criteria).

BOX-PCR genomic fingerprinting. For repetitive DNA PCR fingerprinting we used the BOX A repetitive element originally described by Martin et al. (37). BOX fingerprints were generated by using 30 to 40 ng of genomic DNA and the methods described by Louws et al. (35). The BOX A1R primer (CTACGGCAAGGCGACGCTGACG) was synthesized by Keystone Laboratories, Inc., Menlo Park, Calif., and was used in 50-μl PCR mixtures containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, 10% dimethyl sulfoxide, each deoxynucleoside triphosphate at a concentration of 200 μM, 0.2 μM BOX A1R primer, and 1.25 U of LD-*Taq* (Perkin-Elmer) in a Perkin-Elmer model 9600 thermal cycler. The genomic DNA, primer, and dimethyl sulfoxide were hot-started at 80°C before the *Taq*-buffer-deoxynucleoside triphosphate mixture was added. The PCR protocol included an initial denaturation step for 2 min at 94°C, 30 reaction cycles (94°C for 15 s, 52°C for 15 s, and 65°C for 6 min, with a 5-s extension), and a final 20-min extension step at 65°C prior to soaking at 4°C. Thirty microliters of the amplification products was analyzed by agarose (1.5%) gel electrophoresis in TAE buffer (40 mM Tris-acetate, 2 mM EDTA).

DNA-DNA hybridization. The general approaches used for DNA-DNA hybridization have been described elsewhere (27). Briefly, genomic DNA from each strain was prepared for blotting by shearing in a French press at 16,000 lb/in^2. Approximately 0.5 μg of DNA was blotted onto a nylon membrane (Boehringer-Mannheim, Indianapolis, Ind.) by using a dot blot manifold (Schleicher and Schuell) and methods suggested by the manufacturer. Additional DNA from each strain was labeled to a high specific activity ($>10^8$ dpm/μg) with ^{32}P (by using a Prime-It II random primer labeling kit [Stratagene, La Jolla, Calif.]) and used to probe the immobilized DNA samples on the membranes. Hybridizations were conducted at 70°C in 6× SSC (1× SSC is 0.16 M NaCl plus 0.015 M sodium citrate, pH 7.0) containing Denhardt's solution (10) overnight. The membranes were then washed twice with 2× SSC–0.1% sodium dodecyl sulfate (SDS) for 5 min at room temperature, once with 0.1× SSC–0.1% SDS for 15 min at 70°C, and once with 2× SSC at room temperature. After blotting to remove excess liquid, the radioactivity associated with the individual sample areas on the dot blots was counted by using a Top Count microplate scintillation counter (Packard Instrument Co., Meriden, Conn.). Each hybridization value represents the mean of three replicates. The average range of variation was ±4%.

G+C content of DNA. The G+C contents of the *Sphingomonas* isolates were determined as described by Mesbah and Whitman (40). Analyses were performed with a series II 1090 liquid chromatograph (Hewlett-Packard, Avondale, Pa.) fitted with an Alltech Econosphere C$_{18}$ reversed-phase column (250 mm by 4.6 mm [inside diameter]; particle size, 0.5 μm; Alltech Associates, Inc., Deerfield, Ill.). The mobile phase was 20 mM triethylamine phosphate (pH 5.1) in 12% methanol. The flow rate was 1 ml/min, and the column temperature was 37°C. Nonmethylated lambda phage DNA (500 μg/ml; Sigma) with a known molar ratio of dT to dG was used as a standard. Each reported value represents the mean of five determinations. The standard deviations ranged from 0.23 to 0.93 mol% G+C, and the average standard deviation was 0.52 mol% G+C.

Fatty acid analyses. Analysis of fatty acids was performed as described by Guckert et al. (22). Nutrient broth (BBL) cultures (500 ml) of the various

TABLE 1. Strains included in phylogenetic analysis of 16S rRNA gene sequences

Taxon	Other (former) designation(s)	Culture collection accession no.	Original source of strain	Source of sequence	EMBL and/or GenBank accession no.	Reference
Arthrobacter globiformis		DSM 20124		RDP	M23411	
Blastobacter natatorius				RDP	X73043	24
Brevundimonas diminuta	(*Pseudomonas diminuta* type strain)	ATCC 11568^{T} (= CCEB 513^{T} = CCUG 1427^{T} = LMG 1793^{T})		RDP	M59064	
Caulobacter subvibrioides	Strain CB81			RDP	M83797	50
Erythrobacter longus	Strain Och 101	ATCC 33941^{T} (= IFO 14126^{T})		RDP	M59062	
Erythromicrobium ramosum	Strain E5 Yurkov^{T}, strain Drews^{T}	DSM 8510^{T}		RDP	X72909	65
Hyphomonas jannaschiana	Strain VP-1	ATCC 33882		RDP	M83806	50
Porphyrobacter neustonensis		ACM 2844		RDP	M96745	20
Pseudomonas mendocina		ATCC 25411^{T}		RDP	M59154	
Rhizomonas suberifaciens		IFO 15211^{T}		RDP	D13737	56
Rhodospirillum salexigens		DSM 2132 (= ATCC 35888)		RDP	M59070	
Sphingomonas adhaesiva	(*Pseudomonas paucimobilis* Op-55^{T})	GIFU 11458^{T} (= IFO 15099^{T} = JCM 7370^{T})	"Sterile" water	RDP	D16146	64
Sphingomonas adhaesiva	(*Pseudomonas paucimobilis* Op-55^{T})	JCM 7370^{T} (= IFO 15099^{T} = GIFU 11458^{T})	"Sterile" water	RDP	X72720	41
Sphingomonas adhaesiva	(*Pseudomonas paucimobilis* Op-55^{T})	IFO 15099^{T} (= GIFU 11458^{T} = JCM 7370^{T})	"Sterile" water	RDP	D13722	55
Sphingomonas asaccharolytica	Y-345^{T}	IFO 15499^{T}	Plant roots	GenBank	D28571-D28573	56
Sphingomonas capsulata	(*Flavobacterium capsulatum* type strain)	GIFU 11526^{T} (= IFO 12533^{T} = JCM 7508^{T} = ATCC 14666^{T} = DSM 31096^{T} = NCIB 9890^{T})	Distilled water	RDP	D16147	64
Sphingomonas capsulata	(*Flavobacterium capsulatum* type strain)	ATCC 14666^{T} (= GIFU 11526^{T} = IFO 12533^{T} = JCM 7508^{T} = DSM 31096^{T} = NCIB 9890^{T})	Distilled water	RDP		
Sphingomonas macrogoltabidus	(*Flavobacterium* sp.)	IFO 15033^{T} (= ATCC 51380^{T})		RDP	D13723	55
Sphingomonas mali	Y-347^{T}	IFO 15500^{T}	Plant roots	GenBank	D28574-D28576	56
Sphingomonas parapaucimobilis	(*Pseudomonas paucimobilis* OH 3807)	IFO 15100^{T} (= JCM 7510^{T} = GIFU 11387^{T})	Urine	RDP	D13724	55
Sphingomonas parapaucimobilis	(*Pseudomonas paucimobilis* OH 3807)	JCM 7510^{T} (= IFO 15100^{T} = GIFU 11387^{T})	Urine	RDP	X72721	41
Sphingomonas paucimobilis	(*Pseudomonas paucimobilis*)	IFO 13935^{T} (= JCM 7516^{T} = GIFU 2395^{T} = ATCC 29837^{T} = NCTC 11030^{T})	Respirator	RDP	D13725	55
Sphingomonas paucimobilis	(*Pseudomonas paucimobilis*)	GIFU 2395^{T} (= IFO 13935^{T} = JCM 7516^{T} = ATCC 29837^{T} = NCTC 11030^{T})	Respirator	RDP	D16144	64
Sphingomonas paucimobilis	(*Pseudomonas paucimobilis*)	ATCC 29837^{T} (= IFO 13935^{T} = JCM 7516^{T} = GIFU 2395^{T} = NCTC 11030^{T})	Respirator	GenBank	U20776	16
Sphingomonas paucimobilis	(*Pseudomonas paucimobilis*)	ATCC 29837^{T} (= IFO 13935^{T} = JCM 7516^{T} = GIFU 2395^{T} = NCTC 11030^{T})	Respirator	GenBank	U37337	
Sphingomonas paucimobilis	(*Flavobacterium devorans* type strain)	ATCC 10829 (= NRRL B-54 = GIFU 1367 = JCM 7511)	Not recorded	RDP		
Sphingomonas paucimobilis	Strain EPA 505			GenBank	X94100	
Sphingomonas paucimobilis	Strain EPA 505			GenBank	U37341	
Sphingomonas pruni	Y-250^{T}	IFO 15498^{T}	Plant roots	GenBank	D28568-D28570	56
Sphingomonas rosa	(*Agrobacterium rhizogenes*)	IAM 14222^{T} (= IFO 15208^{T} = NCPPB 2661^{T})	Plant roots	GenBank	D13945	56
Sphingomonas sanguis	(*Sphingomonas* genospecies 1, *Pseudomonas paucimobilis*)	IFO 13937^{T} (= JCM 7514^{T} = GIFU 2397^{T} = NCTC 11032^{T})	Blood	RDP	D13726	55
Sphingomonas subarctica	Strain KF1 (*Pseudomonas* sp.)			GenBank	X94102	44a
Sphingomonas subarctica	Strain KF3 (*Pseudomonas* sp.)			GenBank	X94103	
Sphingomonas subarctica	Strain NKF1 (*Pseudomonas* sp.)			GenBank	X94104	
Sphingomonas terrae	(*Flavobacterium* sp.)	IFO 15098^{T} (= JCM 7513^{T})		RDP	D13727	55
Sphingomonas yanoikuyae	(*Sphingobacterium* sp. strain AB 1105^{T})	IFO 15102^{T} (= JCM 7371^{T} = GIFU 9882^{T} = ATCC 51230^{T})	Clinical specimen	RDP	D13728	55
Sphingomonas yanoikuyae	(*Sphingobacterium* sp. strain AB 1105^{T})	GIFU 9882^{T} (= IFO 15102^{T} = JCM 7371^{T} = ATCC 51230^{T})	Clinical specimen	RDP	D16145	64
Sphingomonas yanoikuyae	(*Sphingobacterium* sp. strain AB 1105^{T})	JCM 7371^{T} (= IFO 15102^{T} = GIFU 2397^{T} = ATCC 51230^{T})	Clinical specimen	RDP	X72725	41
Sphingomonas yanoikuyae	Strain B1 (PAH degrader)[a]			GenBank	X85023	31

Continued on following page

TABLE 1—*Continued*

Taxon	Other (former) designation(s)	Culture collection accession no.	Original source of strain	Source of sequence	EMBL and/or GenBank accession no.	Reference
Sphingomonas yanoikuyae	Strain Q1 (*Pseudomonas paucimobilis*)			GenBank	U37525	60
Sphingomonas yanoikuyae	(*Chromobacterium lividum*)	IAM 14225 (= IFO 15163 = NCTC 10590)	Plant roots	GenBank	D13946	56
Sphingomonas yanoikuyae	B1 (*Beijerinckia* sp. strain B1)			GenBank	U37524	60
Sphingomonas sp.	Strain B0477	SMCC B0477	Subsurface	GenBank	U20772	
Sphingomonas sp.	Strain B0478^{T}	SMCC B0478^{T}	Subsurface	GenBank	U20773	
Sphingomonas sp.	Strain B0522	SMCC B0522	Subsurface	GenBank	U20774	
Sphingomonas sp.	Strain B0695	SMCC B0695	Subsurface	GenBank	U20755	
Sphingomonas sp.	Strain B0712^{T}	SMCC B0712^{T}	Subsurface	GenBank	U20775	
Sphingomonas sp.	Strain F199^{T}	SMCC F199^{T}	Subsurface	GenBank	U20756	
Sphingomonas sp.	Strain A175			GenBank	X94101	
Sphingomonas sp.	Strain B1			GenBank	X94099	
Sphingomonas sp.	Strain BF14 (*Blastobacter* sp.)			RDP	Z23157	24
Sphingomonas sp.	Strain BN6			GenBank	X94098	
Sphingomonas sp.	Strain RW1		River water	RDP	X72723	41
Sphingomonas sp.	Strain SS86			RDP	D16148	
Sphingomonas sp.	Strain SYK6			RDP	D16149	
Sphingomonas sp.	Strain UN1F1			GenBank	U37345	
Sphingomonas sp.	Strain UN1F2			GenBank	U37346	
Sphingomonas sp.	Strain UN1P1			GenBank	U37347	
Strain C7 (azo dye degrading)	(*Pseudomonas paucimobilis*)			RDP	L22759	21
Zymomonas mobilis	*Z. mobilis* subsp. *mobilis*	ATCC 10988^{T} (= NRRL B-806^{T} = NCI 8938^{T})		RDP		

[a] PAH, polycyclic aromatic hydrocarbon.

Sphingomonas strains were incubated at room temperature for 4 days, harvested by centrifugation, and lyophilized. After extraction by a modified Bligh-Dyer procedure (6), the total extractable lipid was divided in half. One-half was fractionated on a silicic acid column, from which the polar lipids were collected and transesterified into methyl ethers for gas chromatography (GC) analysis. The other half of the extractable lipid was subjected to 5% KOH saponification (44). Fatty acid methyl esters were formed by using strong acid methanolysis. The residue from the Bligh-Dyer procedure was subjected to acid hydrolysis and esterification similar to that described by Mayberry and Lane (38). The hydroxy fatty acids were derivatized with *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA), which resulted in trimethylsilyl esters before analysis by GC. Mass spectral verification of all lipid moieties was accomplished by using a model HP5971 mass selective detector interfaced with a model HP5890 series II GC equipped with a Restek Rt$_x$-1 capillary column (length, 60 m; inside diameter, 0.2 mm; film thickness, 0.1 μm). The temperature program used for this analysis was as follows: the initial temperature was 100°C, and the temperature was immediately increased at a rate of 10°C/min to 150°C, held at 150°C for 1 min, and then increased at a rate of 3°C/min to the final temperature, 280°C, which was maintained for an additional 3 min. The mass selective detector was run at 70 eV by using positive ion electron impact ionization.

Sphingolipid analyses. Cells for sphingolipid analyses were obtained from the same lyophilized preparations as those used for phospholipid fatty acid analysis. Lipids from *S. paucimobilis*, *S. capsulata*, and strains B0477, B0478^{T}, B0522, and B0712^{T} were analyzed by the chloroform-methanol extraction method of Kazuyoshi et al. (30). Lipids from strains F199^{T} and B0695 were subjected to the sequential saponification-hydrolysis procedure described by Mayberry and Lane (38). The resulting lipid extracts were spotted onto thin-layer chromatography plates (250-μm-thick type 60A plates obtained from Aldrich Chemical Co., Milwaukee, Wis.), which were subsequently developed with a two-step solvent system (chloroform to 15 cm and then hexane-diethyl ether [35:65, vol/vol]). Sphingoid bases were collected from the origin (between R_f −0.1 and R_f 0.1) and recovered by elution with chloroform-methanol (2:1, vol/vol). The bases were derivatized in BSTFA (Pierce Chemical Co., Rockford, Ill.) to form trimethylsilyl ethers, which were then identified and quantified by GC-mass spectrometry as described above for the phospholipid fatty acids.

Nucleotide sequence accession numbers. The GenBank accession numbers for the 16S rDNA sequences determined in this study are as follows: F199^{T}, U20756; B0477, U20772; B0478^{T}, U20773; B0522, U20774; B0695, U20755; and B0712^{T}, U20775.

RESULTS

Morphological and biochemical characteristics. All of the subsurface strains were non-spore-forming, nonmotile, gram-negative rods. The subsurface strains produced small (1- to 3-mm), yellow colonies on 5% PTYG medium, although the B0695 and B0712^{T} colonies were noticeably lighter in color than the colonies of the other strains. Only strain B0712^{T} produced a visible capsule when it was grown in 10% PTYG broth. The G+C contents of the DNAs were as follows: *S. capsulata* ATCC 14666, 63.7 mol%; F199^{T}, 64.2 mol%; B0522, 62.9 mol%; B0695, 65.0 mol%; B0478^{T}, 60.0 mol%; B0712^{T}, 65.4 mol%; and B0477, 63.1 mol%. The predominant isoprenoid quinone in all strains was ubiquinone Q-10. These morphological and biochemical characteristics are consistent with the characteristics of members of the genus *Sphingomonas* (55, 64), except that the G+C content of strain B0478^{T} is slightly lower than the range specified in the current genus description (61.6 to 67.8 mol%).

Phylogenetic analysis of 16S rRNA gene sequences. Phylogenetic trees depicting the results of distance matrix and parsimony analyses of 16S rRNA gene sequences are shown in Fig. 1 and 2, respectively. Both analyses clearly indicated that the subsurface strains fall in the genus *Sphingomonas*, as it is currently defined (55, 64). Both analyses were also in agreement with respect to the phylogenetic relatedness of the subsurface strains to each other and to the various previously described *Sphingomonas* species that were included in the analysis.

Five of the subsurface strains (B0478^{T}, B0522, B0695, B0712^{T}, and F199^{T}) formed a distinct cluster with *S. capsulata* (Fig. 1 and 2). Three of these strains (B0522, B0695, and F199^{T}) were phylogenetically very closely related and had almost identical 16S rRNA gene sequences (the levels of similarity were 99.8 to 99.9% for 1,292 bases), implying that they may be members of the same species. The levels of sequence similarity between these strains and the two *S. capsulata* species included in the analysis were somewhat lower (97.8 to 98.0%). Moreover, both analytical methods clearly separated the subsurface and *S. capsulata* strains on distinct branches of

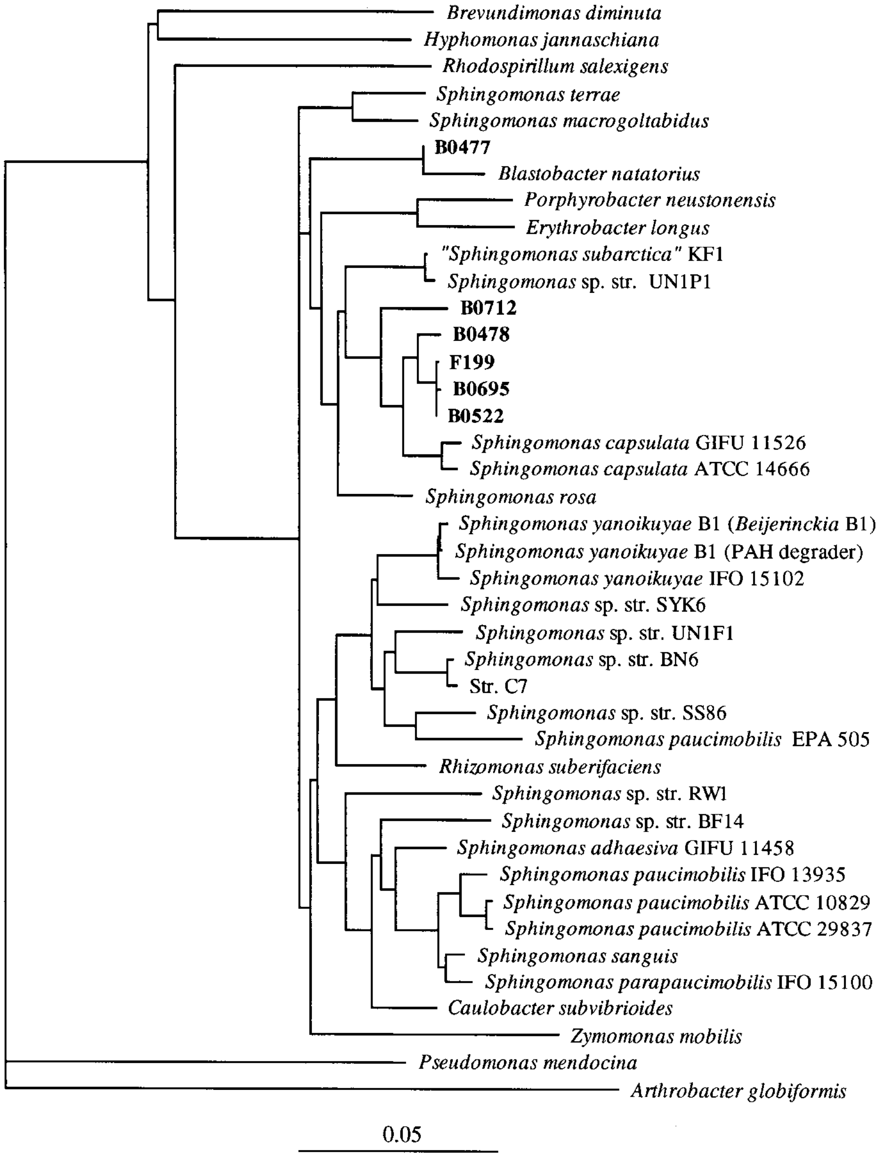

FIG. 1. Phylogenetic tree for six subsurface isolates and selected strains of eubacteria (see Table 1), based on a distance matrix analysis. The PHYLIP program (14) was used to calculate distances by the method of Jukes and Cantor (28), after which the FITCH option was used to estimate phylogenies from distance matrix data. *Arthrobacter globiformis* was used as the outgroup. Scale bar = 5 substitutions per 100 bases.

the trees. These results imply that strains B0522, B0695, and F199^T are not strains of *S. capsulata* and may represent a separate species.

Subsurface strain B0478^T was phylogenetically most closely related to strains B0522, B0695, and F199^T, but was always separated from those strains on a distinct branch of the trees (Fig. 1 and 2). It was not clear from the levels of sequence similarity (98.9 to 99.0%) whether B0478^T might differ from the other three subsurface strains at the species level. On the other hand, B0478^T was as phylogenetically distant from *S. capsulata* as the other three subsurface strains were, and the levels of sequence similarity between it and the *S. capsulata* strains (97.8 to 97.9%) were in a range that some have suggested is too low for members of a single species (7, 11).

Both analytical methods clearly separated subsurface strain B0712^T from all of the strains described above (including *S. capsulata*) and assigned it to a distinct and comparatively deep branch of the tree (Fig. 1 and 2). This finding and the levels of sequence similarity between B0712^T and the strains described above (96.1 to 97.2%) imply that B0712^T is a member of a distinct *Sphingomonas* species.

Subsurface strain B0477 did not cluster with *S. capsulata* and the other subsurface isolates (Fig. 1 and 2). Of the species for which sequences were available from the RDP or GenBank/EMBL, B0477 was most closely related to *Blastobacter natatorius* (level of sequence similarity, 99.1%).

The results of the sequence analyses were in general agreement with the results of previous studies (41, 55–57, 64) with regard to the overall phylogenetic arrangement of the genus *Sphingomonas*. Some of the higher-order branching was not resolved consistently in the bootstrap parsimony analysis (Fig. 2). Nevertheless, the clustering of species and the relative distances were very much as reported previously. For example, the clustering and order of branching for *Sphingomonas adhae-*

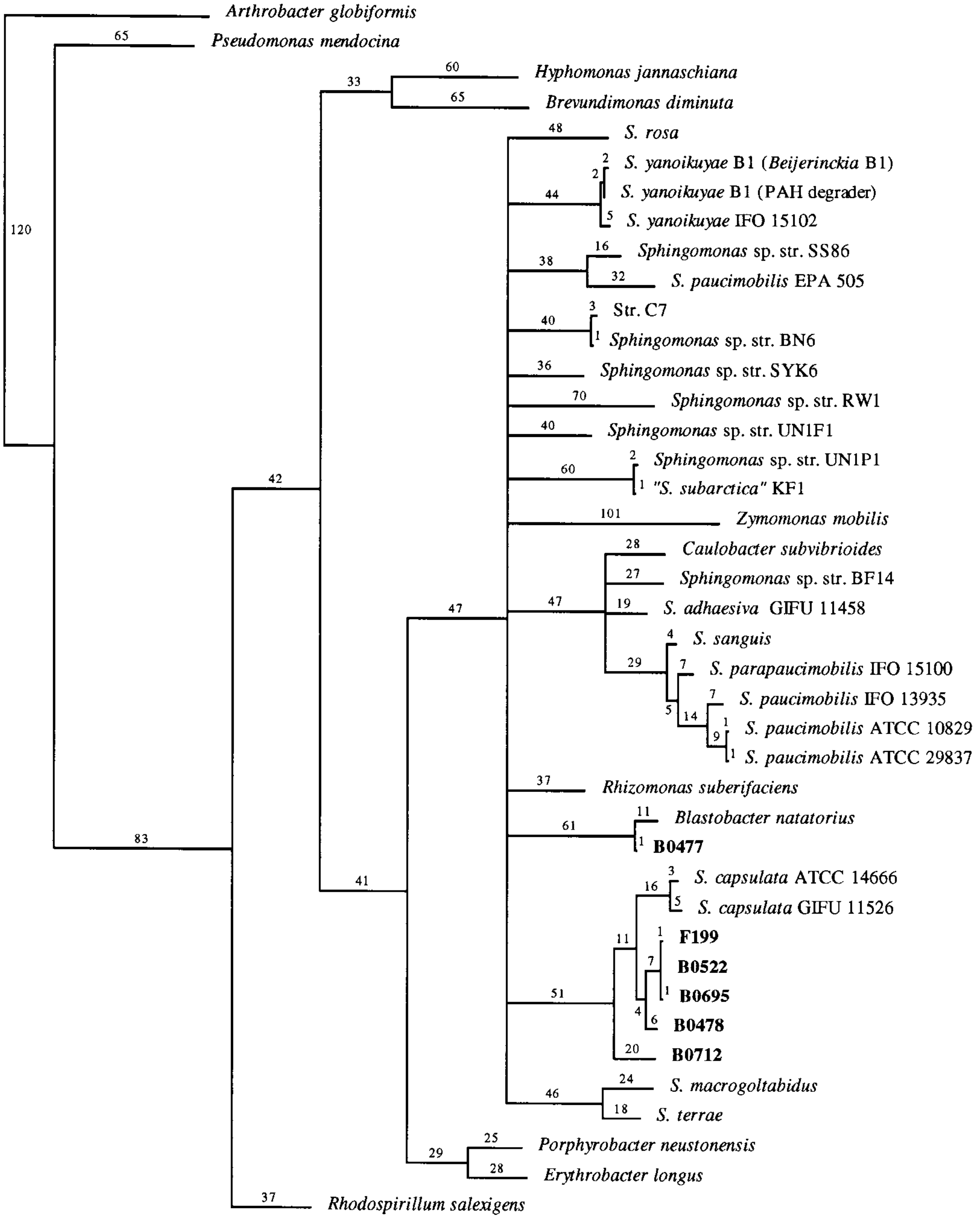

FIG. 2. Consensus phylogenetic tree for six subsurface isolates and selected strains of eubacteria (see Table 1), based on parsimony analysis. The PAUP program (53) was used to analyze 1,252 characters of aligned nucleotide sequences. A heuristic search retained two trees with a minimum length of 1,518 steps that differed only in the branching order of *S. macrogoltabidus* and *S. terrae*. The tree shown was generated by bootstrapping at the greater-than-50% confidence limits, with 100 replications (13). The number above each branch is the branch length. *Arthrobacter globiformis* was used as the outgroup.

siva, *Sphingomonas sanguis*, *S. paucimobilis*, and *Sphingomonas parapaucimobilis*, as well as the clustering and order of branching for *Sphingomonas macrogoltabidus* and *Sphingomonas terrae*, were identical to the clustering and order of branching reported by Takeuchi et al. (56). Interestingly, the subsurface strains did not cluster with *Sphingomonas* isolates that have been reported to degrade dibenzo-*p*-dioxin (RW1), chlorophenol (KF1), or polyaromatic hydrocarbons (e.g., B1, UN1F1, and UN1P1).

DNA-DNA reassociation analysis. Stackebrandt and Goebel (49) recently recommended that DNA-DNA reassociation values be used to assess whether strains are members of a single species when levels of 16S rRNA sequence similarity are 97% or higher. Therefore, a DNA-DNA reassociation analysis was carried out to obtain more definitive information on the various species level relationships among the subsurface strains and *S. capsulata* that were implied by the 16S rRNA gene sequence analysis (see above). The results of this analysis (Table 2) indicated that subsurface strains B0522, B0695, and F199^T had DNA-DNA reassociation values between 60 and 116% (depending on which DNA was used as the probe). The Ad Hoc Committee on Reconciliation of Approaches to Bac-

TABLE 2. DNA-DNA reassociation values for various *Sphingomonas* strains

Isolate	% Reassociation with labeled DNA from:									
	ATCC 51230	ATCC 14666	F199^T	B0522	B0695	B0478^T	B0712^T	B0477	B1	Q1
S. yanoikuyae ATCC 51230	100	4	0	2	1	2	2	1	53	32
S. capsulata ATCC 14666	10	100	3	4	6	6	4	2	11	8
Sphingomonas sp. strain F199^T	13	5	100	73	60	14	7	3	10	6
Sphingomonas sp. strain B0522	5	6	107	100	66	17	8	1	5	4
Sphingomonas sp. strain B0695	8	8	116	98	100	21	10	2	6	9
Sphingomonas sp. strain B0478^T	5	9	16	16	11	100	8	7	3	4
Sphingomonas sp. strain B0712^T	7	9	10	7	10	9	100	3	12	6
Sphingomonas sp. strain B0477	8	5	0	4	3	13	5	100	7	13
S. yanoikuyae B1	53	4	0	1	3	5	2	1	100	44
S. yanoikuyae Q1	31	5	1	1	2	3	0	1	33	100

terial Systematics (61) has suggested that a phylogenetically defined species consists of strains that exhibit approximately 70% or greater DNA-DNA relatedness and a difference in the denaturation temperatures of homoduplexes and heteroduplexes of 5°C or less. Takeuchi et al. (55, 56) have used similar criteria in defining several new species of the genus *Sphingomonas*. Thus, the reassociation values strongly imply that strains B0522, B0695, and F199^T are members of a single species. The reassociation values between these strains and all other strains tested (Table 2) were less than 21%, however, strongly implying that these three strains were distinct from the other strains at the species level.

The DNA-DNA reassociation values between subsurface strain B0478^T and all other strains were no higher than 21%, while the DNA-DNA reassociation values between strain B0712^T and all other strains were no higher than 12%. These results indicate that B0478^T and B0712^T are both highly likely to be members of distinct *Sphingomonas* species.

BOX-PCR genomic fingerprints. Genomic fingerprinting of bacteria based on PCR of repeated sequence regions can be used to determine the degrees of similarity of closely related bacteria and to determine whether similar strains are clonally related (59). The BOX-PCR method has been used successfully to identify and classify *Xanthomonas* and *Pseudomonas* strains (35) and was used in this study to characterize the genomic structure of the subsurface *Sphingomonas* strains. Subsurface *Sphingomonas* strains B0695 and B0522 share the greatest number of bands in the BOX fingerprints (Fig. 3) but are clearly not clonal. F199^T also shares several bands with B0695 and B0522, but all three of these isolates are quite distinct from the *S. capsulata* type strain. The remaining subsurface strains (B0477, B0478^T, and B0712^T) are clearly distinct from each other and from the type strains.

Physiological characteristics. Representative physiological characteristics of *S. paucimobilis*, *S. capsulata*, and the subsurface strains are summarized in Table 3. The physiological traits of the subsurface strains generally matched those of the genus *Sphingomonas*, as defined by Yabuuchi et al. (64) and emended by Takeuchi et al. (55) (catalase positive; oxidative acid production from pentoses, hexoses, and disaccharides, but not from inulin or polyalcohols). The only exception was strain B0477, which failed to produce acid from glucose, maltose, and raffinose.

Subsurface strains B0522, B0695, and F199^T had virtually identical physiological traits, a finding that is consistent with the evidence (from 16S rRNA gene sequence, DNA-DNA reassociation, and BOX-PCR analyses [see above]) that they are members of a single species. Their physiological traits differed from those of *S. capsulata* (the phylogenetically most closely related species of *Sphingomonas*) in several ways, most notably in the ability to grow readily on aromatic compounds such as benzoate, *p*-cresol, and *m*-xylene (Table 3) (16).

The physiological characteristics of subsurface strains B0478^T and B0712^T were quite similar, except for the types of aromatic compounds on which they could grow (Table 3) (16). They differed from *S. capsulata* and *S. paucimobilis* in their ability to grow on aromatic compounds and their failure to aerobically assimilate L-malate or produce acid from galactose. Strains

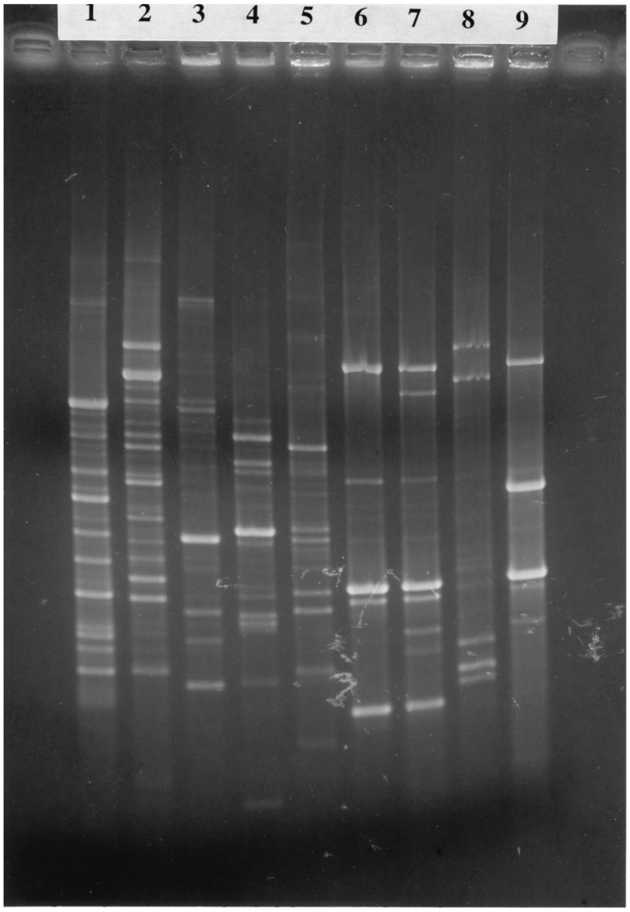

FIG. 3. BOX-PCR fingerprints of F199^T (lane 1), B0712^T (lane 2), B0695 (lane 3), B0522 (lane 4), B0478^T (lane 5), B0477 (lane 6), *S. paucimobilis* ATCC 29837 (lane 7), *S. capsulata* ATCC 14666 (lane 8), and *Burkholderia cepacia* (lane 9).

TABLE 3. Physiological characteristics of *Sphingomonas* strains[a]

Physiological characteristic	*S. paucimobilis* ATCC 29837	*S. capsulata* ATCC 14666	F199^{T}, B0522, and B0695	B0478^{T}	B0712^{T}	B0477	Test basis or reference[b]
N-Acetyl-β-glucosaminidase activity	+	−	+	−	−	−	Z
Aerobic assimilation of:							
L-Arabinose	+	+	−	+/−	+	−	N
Adipate	−	−	+/−	−	−	−	N
D-Gluconate	−	+	−	−	−	−	N
L-Malate	+	+	−	−	−	−	N
Relative growth on the following aromatic compounds[c]:							
Benzoate	−	−	+	+	+	+	16
p-Cresol	−	+	++	−	−	−	16
Naphthalene	−	−	+	+	−	−	16
m-Xylene	−	−	++	−	++	−	16
Oxidative acid production from:							
L-Arabinose	−	+	−	+	+	−	O
L-Fucose	−	+	−	−	−	−	C
Galactose	+	+	−	−	−	−	C
Glucose	+	+	+	+	+	−	O, C
Maltose	+	+	+	+	+	−	C
Raffinose	+	+	+	+	+/−	−	O, C
Sucrose	+	+	+	+	+	+/−	O, C
Trehalose	−	+	−	−	−	−	O
D-Xylose	+	+	+	+	−	−	C

[a] All strains were positive for catalase and esculin hydrolysis. All strains were negative for glucose fermentation; indole production; urease; gelatin hydrolysis; arginine dihydrolase; and oxidative acid production from D-arabinol, L-arabinol, dulcitol, inulin, mannitol, and sorbitol.

[b] C, API 50CH; O, Difco OF medium; N, API NFT; Z, API ZYM (see Materials and Methods).

[c] These plasmid-encoded traits are included for comparison only; they are not presented as defining taxonomic traits.

B0478^{T} and B0712^{T} differed from strains B0522, B0695, and F199^{T} in growth on certain aromatic compounds, aerobic assimilation of L-arabinose, and oxidative acid production from L-arabinose.

Chemical characteristics. Sphingolipids were present in all of the subsurface strains, although there were differences among the strains with respect to the relative amounts of specific lipids (16). The predominant long-chain bases in F199^{T} and B0695 were cy21:0 and 18:0, whereas cy20:0 and 18:0 were the predominant bases in strains B0712^{T}, B0522, B0478^{T}, and B0477. The predominant sphinganine bases in the *S. paucimobilis* and *S. capsulata* type strains were 18:0 and cy21:0.

In all of the subsurface *Sphingomonas* strains and in the *S. capsulata* and *S. paucimobilis* type strains, the major hydroxy fatty acid was 2OH14:0 (Table 4). However, there were a number of differences in the relative amounts of minor hydroxy fatty acids among the various strains. For example, the subsurface strains generally had greater amounts of 2OH15:0, 2OH16:0, and 2OH17:1 than the type strains, whereas the *S. capsulata* type strain had a greater amount of 2OH14:1 than the other strains. Of all of the strains tested, B0477 was the most different in terms of its hydroxy fatty acid profile.

There were also several differences in the relative amounts of total lipid fatty acid components in the various strains tested (Table 5). For example, 18:1ω5c, 18:0, and br19:1 were present in F199^{T}, B0695, and B0522, but were not detected in the *S. capsulata* type strain. Among the strains tested, B0477 had the most unusual total lipid fatty acid profile.

The lipid compositions of all of the subsurface strains fit the current description of the genus *Sphingomonas* (55) in that 18:1 was a major constituent of the cellular lipids, the predominant hydroxy fatty acids were 2OH14:0 and 2OH15:0, and long-chain sphinganine bases were present. In addition to differences in the lipid and fatty acid compositions of the cellular membranes among the various subsurface strains, there were several substantial differences between the subsurface strains and *S. capsulata* and *S. paucimobilis*. Strains F199^{T}, B0695, and B0522 contained between 9.8 and 35.4 mol% br19:1, whereas this fatty acid was not detected in the two type strains (Table 5). Strain B0478^{T} contained 4.3 mol% br19:1, but B0712^{T} and B0477 did not contain detectable levels of this fatty acid. Also, the subsurface strains contained small amounts of several odd-numbered hydroxy fatty acids (including 2OH13:0 and 2OH17:1) that were not detected in the type strains (Table 4).

DISCUSSION

The morphological, physiological, and biochemical characteristics of subsurface strains B0478^{T}, B0522, B0695, B0712^{T}, and F199^{T} are consistent with the characteristics of the genus *Sphingomonas*, as defined by Yabuuchi et al. (64) and emended by Takeuchi et al. (55). The G+C contents of strains B0522, B0695, B0712^{T}, and F199^{T} (62.9 to 65.4 mol%) were well

TABLE 4. Levels of hydroxy fatty acids in strain ATCC 14666 and subsurface *Sphingomonas* strains

Fatty acid	mol% in:						
	S. capsulata ATCC 14666	F199^{T}	B0552	B0695	B0478^{T}	B0712^{T}	B0477
2OH12:0	0.8	2.1	0.9	2.0	0.8	1.0	0.1
2OH13:0	0.0	1.8	1.8	0.7	2.3	2.4	0.1
2OH14:1	3.6	0.5	0.6	0.0	0.2	0.7	0.0
2OH14:0	86.1	84.6	77.4	93.1	75.8	79.8	34.4
2OH15:0	1.8	5.9	8.8	2.5	13.3	3.5	11.5
18:1/2OH16:1	1.5	0.4	3.0	0.2	1.2	5.4	2.6
18:0/2OH16:1	5.7	2.1	4.7	0.3	3.6	6.3	26.4
2OH16:0	0.5	1.4	1.3	0.9	2.2	0.6	24.2
2OH17:1	0.0	0.5	0.7	0.3	0.5	0.2	0.5
2OH18:1	0.0	0.7	0.9	0.0	0.1	0.1	0.2

TABLE 5. Levels of total lipid fatty acids in strain ATCC 14666 and subsurface *Sphingomonas* strains

Polar lipid fatty acid	mol% in:						
	S. capsulata ATCC 14666	F199^T	B0522	B0695	B0478^T	B0712^T	B0477
2OH12:0	2.0	0.0	1.6	0.0	0.4	0.8	0.1
14:0	0.7	1.1	0.3	0.1	0.7	2.5	0.0
Unknown 1	9.0	0.6	2.3	0.2	1.0	1.2	0.5
2OH14:0	47.8	26.7	39.5	24.5	36.7	38.8	177
16:1ω7c	0.9	2.8	2.1	2.2	2.3	3.1	2.2
16:1ω5c	0.0	0.3	0.2	0.1	0.2	1.3	0.8
16:0	1.7	3.3	1.3	6.4	2.3	2.3	5.7
2OH15:0	0.0	2.2	3.9	1.2	5.0	3.0	2.1
17:1ω6c	0.7	1.1	1.0	0.5	2.2	1.6	3.0
2OH16:1	1.0	2.0	2.7	0.6	1.3	1.8	0.5
17:0/2OH16:1	3.9	1.3	3.9	1.0	3.2	7.8	20.0
2OH16:0	0.0	1.1	0.8	1.0	1.6	1.0	2.5
18:1ω7c	32.5	38.0	28.4	20.9	35.8	31.3	42.3
18:1ω5c	0.0	1.5	0.6	1.2	1.5	3.0	1.9
18:0	0.0	0.6	1.2	1.4	1.4	0.5	0.9
br19:1	0.0	16.6	9.8	35.4	4.3	0.0	0.0
cy19:0	0.0	0.8	0.5	3.3	0.0	0.0	0.0

within the range of values reported for established *Sphingomonas* species (61.7 to 67.8 mol%) (56). The only exception was strain B0478^T (G+C content, 60.0 mol%), but in phylogenetic analyses of 16S rRNA sequences (Fig. 1 and 2), this isolate consistently clustered with *S. capsulata* and the other subsurface strains (except B0477). Moreover, strain B0478^T (like the other subsurface strains) contained ubiquinone Q-10 as the predominant isoprenoid quinone. Based on these findings, we conclude that subsurface strains B0478^T, B0522, B0695, B0712^T, and F199^T are members of the genus *Sphingomonas*, as it is currently defined. The taxonomic status of strain B0477 is not clear because some of its physiological traits are not consistent with the physiological traits of the genus *Sphingomonas* and phylogenetic analyses indicated that it was most closely related to *B. natotorius*. Additional studies will be needed to fully classify this isolate.

On the basis of physiological characteristics, the results of DNA-DNA reassociation studies, and 16S rRNA gene sequence analysis, we propose the following three new species of the genus *Sphingomonas*: *Sphingomonas aromaticivorans* (for strains B0522, B0695, and F199^T [SMCC F199^T]), *Sphingomonas subterranea* (type strain, B0478 [SMCC B0478]), and *Sphingomonas stygia* (type strain, B0712).

The analysis of 16S rRNA gene sequences placed the proposed new *Sphingomonas* species in a well-defined cluster with *S. capsulata*. In 1993, van Bruggen et al. (58) suggested that *S. capsulata* should be transferred to a new genus and that *S. yanoikuyae* should be placed in the genus *Rhizomonas*. Based on the results of a phylogenetic analysis of 16S rRNA gene sequences, Takeuchi et al. (57) agreed with these suggestions. More recently, Takeuchi et al. (56) suggested that the genus *Sphingomonas* sensu stricto should be restricted to the species *S. paucimobilis*, *S. parapaucimobilis*, *S. sanguis*, and *S. adhaesiva* and that all other species should eventually be transferred to other genera. A revision of the genus *Sphingomonas* was beyond the scope of this study, but if *S. capsulata* is moved to a separate genus, as has been suggested, the results of our 16S rRNA gene sequence analyses indicate that the proposed new subsurface species may also be members of that genus.

The new *Sphingomonas* species are described below.

Description of *Sphingomonas aromaticivorans* sp. nov. *Sphingomonas aromaticivorans* (a.ro.ma.ti.ci′vo.rans. N. L. n. *aromaticus*, aromatic compound; L. part. *vorans*, eating; *aromaticivorans*, eating aromatic compounds) is a gram-negative, nonsporing, nonmotile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and yellow. Indole, urease, and arginine dihydrolase are not produced. Catalase positive. Gelatin is not liquefied, and glucose is not fermented. Adipate and esculin are assimilated aerobically, but L-arabinose, D-gluconate, and L-malate are not assimilated aerobically. Acid is produced oxidatively from glucose, maltose, raffinose, sucrose, and D-xylose, but not from L-arabinose, D- or L-arabitol, dulcitol, L-fucose, inulin, mannitol, sorbitol, or trehalose.

The G+C content of the DNA is 62.9 to 65.0 mol%. The major isoprenoid quinone is ubiquinone Q-10. The major nonpolar fatty acids are 18:ω17c, br19:1, 16:0, 16:1ω7c, and 17:1ω6c, and the major 2-hydroxy fatty acids are 2OH14:0 and 2OH15:0. Sphingolipid is present. Source: isolated from saturated Atlantic coastal plain terrestrial subsurface sediments.

The type strain is F199 (= SMCC F199), and reference strains include strains B0522 (= SMCC B0522) and B0695 (= SMCC B0695).

Description of *Sphingomonas subterranea* sp. nov. *Sphingomonas subterranea* (sub.ter.ra′ne.a. L. adj. *subterraneus*, *-a*, underground, subterranean) is a gram-negative, nonsporing, nonmotile, rod-shaped organism. Colonies are circular, entire, low convex, dry, smooth, opaque, and yellow. Indole, urease, and arginine dihydrolase are not produced. Catalase positive. Gelatin is not liquefied, and glucose is not fermented. Esculin is assimilated aerobically, but adipate, D-gluconate, and L-malate are not assimilated aerobically. Acid is produced oxidatively from L-arabinose, glucose, maltose, raffinose, sucrose, and D-xylose, but not from D- or L-arabitol, dulcitol, L-fucose, galactose, inulin, mannitol, sorbitol, or trehalose.

The G+C content of the DNA is 60.0 mol%. The major isoprenoid quinone is ubiquinone Q-10. The major nonpolar fatty acids are 18:1ω7c, br19:1, 16:0, 16:1ω7c, and 17:1ω6c, and the major 2-hydroxy fatty acids are 2OH14:0 and 2OH15:0. Sphingolipid is present. Source: isolated from saturated Atlantic coastal plain terrestrial subsurface sediments.

The type strain is B0478 (= SMCC B0478).

Description of *Sphinogomonas stygia* sp. nov. *Sphingomonas stygia* (sty′gi.a. L. masc. n. *Styx*, underworld river in classical Greek mythology. L. adj. *stygius*, *-a*, pertaining to the underworld, subterranean) is a gram-negative, nonsporing, nonmotile, rod-shaped organism. Colonies are circular, entire, low convex, smooth, opaque, and yellow. Indole, urease, and arginine dihydrolase are not produced. Catalase positive. Gelatin is not liquified, and glucose is not fermented. Esculin and L-arabinose are assimilated aerobically, but adipate, D-gluconate, and L-malate are not assimilated aerobically. Acid is produced oxidatively from L-arabinose, glucose, maltose, and sucrose, but not from D- or L-arabitol, dulcitol, L-fucose, galactose, inulin, mannitol, sorbitol, trehalose, or D-xylose.

The G+C content of the DNA is 65.4 mol%. The major isoprenoid quinone is ubiquinone Q-10. The major nonpolar fatty acids are 18:1ω7c, 16:1ω7c, 18:1ω5c, 14:0, and 16:0, and the major 2-hydroxy fatty acids are 2OH14:0, 18:0/2OH16:1, 18:1/2OH16:1, and 2OH15:0. Sphingolipid is present. Source: isolated from saturated Atlantic coastal plain terrestrial subsurface sediments.

The type strain is B0712 (= SMCC B0712).

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