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Aromatic-Degrading *Sphingomonas* Isolates from the Deep Subsurface

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An obligately aerobic chemoheterotrophic bacterium (strain F199) previously isolated from Southeast Coastal Plain subsurface sediments and shown to degrade toluene, naphthalene, and other aromatic compounds (J. K. Fredrickson, F. J. Brockman, D. J. Workman, S. W. Li, and T. O. Stevens, Appl. Environ. Microbiol. 57:796–803, 1991) was characterized by analysis of its 16S rRNA nucleotide base sequence and cellular lipid composition. Strain F199 contained 2-OH14:0 and 18:1ω7c as the predominant cellular fatty acids and sphingolipids that are characteristic of the genus *Sphingomonas*. Phylogenetic analysis of its 16S rRNA sequence indicated that F199 was most closely related to *Sphingomonas capsulata* among the bacteria currently in the Ribosomal Database. Five additional isolates from deep Southeast Coastal Plain sediments were determined by 16S rRNA sequence analysis to be closely related to F199. These strains also contained characteristic sphingolipids. Four of these five strains could also grow on a broad range of aromatic compounds and could mineralize [^{14}C]toluene and [^{14}C]naphthalene. *S. capsulata* (ATCC 14666), *Sphingomonas paucimobilis* (ATCC 29837), and one of the subsurface isolates were unable to grow on any of the aromatic compounds or mineralize toluene or naphthalene. These results indicate that bacteria within the genus *Sphingomonas* are present in Southeast Coastal Plain subsurface sediments and that the capacity for degrading a broad range of substituted aromatic compounds appears to be common among *Sphingomonas* species from this environment.

Interest in the microbial ecology of terrestrial subsurface environments and the role of microorganisms in subsurface geochemical processes has grown rapidly since it was found that a variety of shallow (16) and deep (1, 4, 6, 30) environments contained substantial numbers of viable microorganisms. There has also been increased interest in utilizing the metabolic activities of the endogenous microbiota for in situ bioremediation of contaminated subsurface sediments and groundwater.

An obligately aerobic, chemoheterotrophic bacterium designated strain F199 was isolated from a Southeast Coastal Plain sediment sample taken from the Middendorf Formation (at a depth of 410 m below land surface) in borehole C10, located near the town of Allendale, S.C., in 1988 (13). Strain F199 was unusual in that it was found to utilize toluene, naphthalene, dibenzothiophene, salicylate, benzoate, *p*-cresol, and all isomers of xylene as sole carbon and energy sources (14). Originally thought to be a gram-positive organism, F199 has since been shown by transmission electron microscopy of thin sections to have a cell wall structure that is typical of gram-negative bacteria (12a).

During a recent 16S rRNA nucleotide base sequence analysis of selected Southeast Coastal Plain sediment isolates in the U.S. Department of Energy (DOE) Subsurface Microbial Culture Collection (3), several additional strains were found to be closely related phylogenetically to F199. The purpose of this study was twofold: (i) to determine the phylogenetic relatedness of F199 and closely related subsurface isolates to established microbial taxa, and (ii) to determine whether the subsurface strains related to F199 also have the ability to degrade a broad range of aromatic compounds.

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MATERIALS AND METHODS

Isolation of bacteria and culture maintenance. The subsurface bacterial strains examined in this study (Table 1) were isolated from deep saturated Southeast Coastal Plain sediments on 1% PTYG medium as described previously (4, 13). All strains were routinely cultured on 1% PTYG, Kings medium B, or mineral salt broth (MSB) (34) containing 5 mM each lactate, acetate, and malate (LAM). When solid media were used, agar was incorporated at 15 g/liter. Stocks of all cultures are maintained in 40% glycerol at −80°C at Pacific Northwest Laboratory and in 7% dimethyl sulfoxide at −75°C in the DOE Subsurface Microbial Culture Collection at Florida State University (3).

16S ribosomal DNA (rDNA) sequencing. Genomic DNA was isolated from *Sphingomonas paucimobilis* (formerly *Pseudomonas paucimobilis*) ATCC 29837 and the subsurface strains by a standard chloroform-isoamyl alcohol extraction procedure (20). Twenty nanograms of DNA was then used as a template for PCR amplification (32) of an approximately 1,500-base segment of the 16S rRNA gene (i.e., nearly the entire gene). The PCR amplification primers were fD1 (AGAGTTTGATCCTGGCTCAG) and rP2 (ACGGCTACCTTGTTACGACTT) (39).

The PCR amplification products were sequenced with an Applied Biosystems model 373A DNA sequencer by the *Taq* DyeDeoxy terminator cycle sequencing method (2, 27). The following primers were used for sequencing *S. paucimobilis* ATCC 29837 and subsurface strains F199 and B0695: C (ACGGGCGGTGTGTAC [24]), corresponding to positions 1406 to 1392 in the 16S rDNA nucleotide sequence for *Escherichia coli* (7); H (ACACGAGCTGACGACAGCCA; *E. coli* positions 1075 to 1056); G (CCAGGGTATCTAATCCTGTT; *E. coli* positions 800 to 781); and A (GTATTACCGCGG[C/G]TGCTG; *E. coli* positions 536 to 519). The resulting sequences were assembled to produce 1,195-base contiguous rDNA sequences corresponding to *E. coli* positions 146 to 1380. Approximately 40% of each contiguous sequence could be read from more than one primer during assembly. Primers C and H were used to sequence strains B0477, B0478, B0522, and B0712. The resulting sequences were assembled to produce 578-base contiguous rDNA sequences corresponding to *E. coli* positions 802 to 1380. The GenBank accession numbers for the assembled 16S rDNA sequences are as

TABLE 1. List of strains

Organism	Strain[a]	Source	Borehole[b]	
			No.	Depth (m)
Sphingomonas capsulata	ATCC 14666	Distilled water	NA[c]	NA
Sphingomonas paucimobilis	ATCC 29837	Hospital respirator	NA	NA
Subsurface *Sphingomonas* sp.	SMCC F199	Middendorf Formation	C10	407
	SMCC B0477	Pee Dee Formation	P24	180
	SMCC B0478	Pee Dee Formation	P24	180
	SMCC B0522	Black Creek Formation	P24	204
	SMCC B0695	Middendorf Formation	P24	259
	SMCC B0712	Middendorf Formation	P24	259

[a] SMCC, U.S. Department of Energy Subsurface Microbial Culture Collection at Florida State University.
[b] For information on borehole P24, see reference 4; for information on borehole C10, see reference 13.
[c] NA, not applicable.

follows: strain F199, accession number U20756; B0477, U20772; B0478, U20773; B0522, U20774; B0695, U20755; B0712, U20775; and *S. paucimobilis* ATCC 29837, U20776.

Analysis of 16S rDNA sequence data. The assembled 16S rDNA sequences for *S. paucimobilis* ATCC 29837 and subsurface strains F199 and B0695 were converted to the equivalent 16S rRNA sequences and hand aligned to several prealigned sets of corresponding rRNA sequences for selected species of eubacteria (see below). The prealigned sets of eubacterial sequences were obtained from the Ribosomal Database Project (RDP) (25). Each set of aligned sequences was analyzed for maximum parsimony with the PAUP program (Phylogenetic Analysis Using Parsimony, Macintosh version 3.0) (36) to construct the most parsimonious phylogenetic tree. Only the phylogenetically informative sites were considered, and alignment gaps were retained in the analysis. A heuristic search was carried out first (with the standard program defaults), after which a bootstrap analysis was used to place confidence limits on the branch points of the resulting phylogenetic trees. Consensus phylogenetic trees for each alignment set were produced by bootstrapping at the greater-than-50% confidence limit, with 100 replications (10).

The phylogenetic positions of *S. paucimobilis* ATCC 29837 and subsurface strains F199 and B0695 were determined by analyzing their 16S rRNA sequences as described above after aligning them with sets of corresponding sequences for increasingly specific groups of eubacteria. The selection of comparison sequences for each successive alignment was based on the analytical results of the previous alignment. The first alignment included representative species from the 16 major taxonomic groups in the RDP database (e.g., spirochetes, purple bacteria, and gram-positive phylum, etc.). Analysis of this alignment with the PAUP program assigned *S. paucimobilis* ATCC 29837 and strains F199 and B0695 to the purple bacteria (member of the class *Proteobacteria*; data not shown). Analysis of an alignment including representative species from the five major subdivisions of the *Proteobacteria* placed the three strains in the alpha subdivision of the phylum (data not shown), after which analysis of an alignment including representative species from the seven subgroups in the alpha subdivision of *Proteobacteria* indicated that they were most closely related to the *Zymomonas* group (group 4; data not shown). The phylogenetic positions of *S. paucimobilis* and strains F199 and B0695 within the *Zymomonas* group were then determined by analysis of an alignment that included selected sequences from this group in the current version (19 June 1994) of the RDP database. (Duplicate or near-duplicate sequences were not included in this alignment.) The final sequence alignment was also analyzed by the distance matrix method with the PHYLIP package of microcomputer programs (11). Distances were calculated by the method of Jukes and Cantor (21), after which phylogenies were estimated with the FITCH option (which makes use of the Fitch-Margoliash criterion [12] and some related least-squares criteria). The positions of F199, B0695, and *S. paucimobilis* relative to the comparison species in the resulting tree (data not shown) were essentially the same as those in the tree produced by parsimony analysis (i.e., by the PAUP program as described above).

The comparatively short 16S rDNA sequences for strains B0477, B0478, B0522, and B0712 were not analyzed with the PAUP program in this study. Instead, these sequences were aligned to the corresponding portions of the sequences for strains F199 and B0695. Sequence similarities among the subsurface isolates were then calculated as an approximate indicator of their relatedness.

Phospholipid fatty acid analyses. Analysis of phospholipid fatty acids was performed as described by Guckert et al. (17). After nutrient broth (BBL) cultures (500 ml) of the various *Sphingomonas* strains were incubated at room temperature for 4 days, they were harvested by centrifugation and lyophilized. After extraction by a modified Bligh and Dyer procedure, 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 analysis. The other half of the extractable lipid was subjected to a 5% KOH saponification (29). Fatty acid methyl ethers were formed by a strong acid methanolysis. The residue from the Bligh and Dyer procedure was subjected to acid hydrolysis and esterification similar to that described by Mayberry and Lane (26). The hydroxy fatty acids were derivatized with BSTFA [*N*,*O*-bis(trimethylsilyl)trifluroacetamide], resulting in trimethylsilyl ethers, before analysis by gas chromatography. Mass spectral verification of all lipid moieties was accomplished with a HP5971 mass selective detector interfaced with a HP5890 series II gas chromatograph equipped with a Restek Rt_x-1 capillary column (60 m by 0.2 mm [inside diameter]; film thickness, 0.1 μm). The temperature program for this analysis was as follows: the initial temperature of 100°C was immediately ramped by 10°C/min to 150°C, held at 150°C for 1 min, and then increased at a rate of 3°C/min to a final temperature of 280°C, which was maintained for an additional 3 min. The mass selective detector was run at 70 eV with positive ion electron impact ionization.

Sphingolipid analyses. Cells for sphingolipid analyses were from the same lyophilized preparations as those used for PLFA analysis. Lipids from *S. paucimobilis*, *Sphingomonas capsulata*, and strains B0477, B0478, B0522, and B0722 were analyzed by the chloroform-methanol extraction method of Kazuyoshi et al. (22). Lipids from strains F199 and B0695 were subjected to sequential saponification-hydrolysis as described by Mayberry and Lane (26). The resulting lipid extracts were spotted onto thin-layer chromatography plates (250-μm-thick model 60Å plates; Aldrich Chemical Co., Milwaukee, Wis.), which were subsequently developed in a two-step solvent system (with chloroform to 15 cm and then hexane-diethyl ether [35:65, vol/vol]). Sphingoid bases were collected from the origin (between an R_f of −0.1 to 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 gas chromatography-mass spectrometry as described above for the phospholipid fatty acid analysis.

Growth on and mineralization of aromatic compounds. *Sphingomonas* cultures were maintained on LAM. Cultures were spotted onto mineral salt (32) agar plates containing LAM that had been sprayed with ethereal solutions of biphenyl (1 M), fluorene (5%), or dibenzothiophene (100 mM) and incubated at 30°C for 11 days. Zones of clearing around individual colonies or production of colored metabolites was used as an indicator of the ability of an organism to degrade the compound. To assess the growth of the strains on other aromatic compounds, MSB cultures without LAM were started with a 1/50 dilution of the MSB-LAM culture stock. Stocks of the aromatic compounds were prepared in dimethyl formamide and added to the cultures at a final concentration of 2 mM. Salicylate and benzoate were prepared in water and added at the same concentration. Controls consisted of MSB alone or MSB supplemented with dimethyl formamide. MSB cultures were incubated at 30°C for 32 days.

[U-^{14}C]toluene (56.3 mCi/mmol) or [1-^{14}C]naphthalene (8.0 mCi/mmol) (Sigma) was used to determine whether the various *Sphingomonas* strains could mineralize these aromatic compounds. Mineralization of naphthalene and toluene was conducted as described previously (14). Briefly, labeled toluene (0.21 μCi) or naphthalene (0.18 μCi) was added to 10 ml of MSB containing approximately 10^8 cells of the various cultures per ml in 150-ml sterile glass dilution bottles. Traps containing 1.0 ml of 1.0 M KOH were suspended in each bottle. Duplicate bottles were incubated at room temperature. Traps were removed at specific time points during incubation, and the amount of radioactivity in the KOH traps was determined by liquid scintillation counting.

RESULTS AND DISCUSSION

Analysis of 16S rRNA sequences. When their 16S rRNA sequences were aligned with the corresponding sequences for selected groups of eubacteria and analyzed by the maximum parsimony method with the PAUP program (36), it was found that subsurface isolates F199 and B0695 fell within the *Zy-*

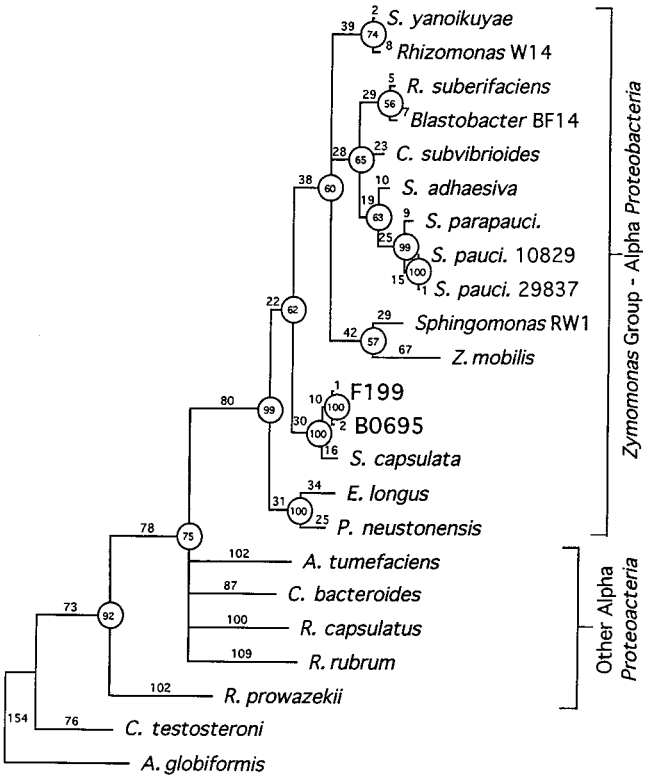

FIG. 1. Consensus phylogenetic tree for *S. paucimobilis* ATCC 29837, two aromatic-compound-degrading subsurface isolates (F199 and B0695), and 20 selected strains of eubacteria. The PAUP program (36) was used to analyze 1,268 characters of aligned nucleotide sequence (corresponding to positions 146 to 1380 in the *E. coli* 16S rRNA gene sequence [7]) for these 23 strains. Sequences for the eubacterial species were obtained from the RDP database (25). The heuristic search retained a single tree with a minimum length of 1,454 steps. The tree shown here was generated by bootstrapping at the greater-than-50% confidence limit, with 100 replications (10). The number above or below each branch is the corresponding branch length, and the numbers in circles indicate the number of trees (of 100 replications) in which the branch point was retained. The eubacterial species included for comparison to the subsurface isolates (as they appear from top to bottom in the tree) were as follows: *Sphingomonas yanoikuyae* (JCM 7371), *Rhizomonas* sp. strain W14, *Rhizomonas suberifaciens* CA1T, *Blastobacter* sp. strain BF14, *Caulobacter subvibrioides* CB81, *Sphingomonas adhaesiva* (JCM 7370), *Sphingomonas parapaucimobilis* (JCM 7510), *S. paucimobilis* (ATCC 10829 = NRRL B-54), *S. paucimobilis* (ATCC 29837), *Sphingomonas* sp. strain RW1, *Zymomonas mobilis* subsp. *mobilis* (ATCC 10988 = NRRL B-806 = NCIB 8938), *S. capsulata* (ATCC 14666 = DSM 31096 = NCI 9890 = JCM 7508), *Erythrobacter longus* Och 101 (ATCC 33941 = IFO 14126), *Porphyrobacter neustonensis* (ACM 2844), *Agrobacterium tumefaciens* (ATCC 4720 = DSM 30150 = CCM 1000 = IAM 1524 = NCIMB 8150), *Caulobacter bacteroides* CB7, *Rhodobacter capsulatus* B10 (ATCC 33303), *Rhodospirillum rubrum* ATH 1.1.1; S.1 (ATCC 1170 = NCIMB 8255), *Rickettsia prowazekii* Breinl (ATCC VR 142), *Comamonas* (*Pseudomonas*) *testosteroni* Rh 1104 (ATCC 11996 = NCIB 8955 = NCTC 10698 [a member of the beta subdivision of *Proteobacteria* included for comparison]), and *Arthrobacter globiformis* (DSM 20124 [a high-G+C gram-positive eubacterium used as the outgroup]). (Note that strains F199, B0695, and *S. paucimobilis* ATCC 29837 were assigned to the same phylogenetic positions in a tree produced by the analysis of these data by a distance matrix method [see Materials and Methods].)

momonas group (group 4) of the alpha subdivision of *Proteobacteria* (see Materials and Methods). The F199 and B0695 sequences were then aligned with representative sequences for members of the *Zymomonas* group and analyzed with the PAUP program. The heuristic search generated a single tree with a minimum length of 1,454 steps. The relative support for each node was determined with a bootstrap analysis (10), which produced the consensus phylogenetic tree shown in Fig. 1.

TABLE 2. 16S rRNA sequence similarities among aromatic compound-degrading subsurface strains

Strain	% Similarity[a] to sequence from strain:					
	F199	B0695	B0522	B0712	B0478	B0477
F199	100	98.8	98.8	97.2	97.1	91.0
B0695		100	99.6	97.8	97.9	91.5
B0522			100	98.1	98.1	91.9
B0712				100	98.4	91.9
B0478					100	91.5
B0477						100

[a] Percentage of identical bases over a 578-base segment of the 16S rRNA gene sequence that could be compared for all strains; uncalled bases (N's) were counted as matching those of the compared sequence.

The sequences for strains F199 and B0695 were nearly identical (99.4% sequence similarity over 1,195 bases). As a result, these two strains clustered together during parsimony analysis and were separated only by very short branch lengths in the resulting phylogenetic tree (Fig. 1). F199 and B0695 fell within a larger cluster containing all species of the genera *Sphingomonas* and *Rhizomonas* that were included in the sequence alignment. Takeuchi et al. (38) suggested recently that the definitions of the genera *Sphingomonas* and *Rhizomonas* need to be revised because the species within them are phylogenetically interrelated. Strains F199 and B0695 specifically clustered with and were most closely related to *S. capsulata*, but they were separated from this species on a distinct branch. Both subsurface isolates appeared to fall well within the phylogenetic bounds of the genus *Sphingomonas* as it is currently defined, but their sequence similarities to *S. capsulata* (97.1 and 97.5% for F199 and B0695, respectively) were low enough (5, 9) to suggest their placement in a separate species.

16S rRNA sequence similarities (over 578 bases examined) among the six subsurface isolates were determined to obtain a rough indication of how closely these strains are related phylogenetically (Table 2). Similarities among strains F199, B0478, B0522, B0695, and B0712 ranged from 97.1 to 99.6%, indicating that these isolates are all quite closely related. The exception was strain B0477, which seemed to be relatively distant from the other isolates (highest sequence similarity, 91.9%).

Analysis of sphingolipids and fatty acids. The presence of sphingolipids is a defining characteristic of the genus *Sphingomonas* (41). Sphingolipids were detected in all of the strains examined in this study (Table 3), thereby corroborating the phylogenetic evidence (from 16S rRNA sequence analysis, described above) that the subsurface isolates are *Sphingomonas*

TABLE 3. Abundance of hydroxy fatty acids and sphinganine bases in the various *Sphingomonas* strains

Organism	Total lipid fatty acids (mol%)			Sphinganine bases (relative abundance)		
	2-OH14:0	2-OH15:0	18:1ω7c	18:0	cy20:0	cy21:0
S. capsulata	47.8	0.0	32.5	+++	−	+++
S. paucimobilis	44.3	0.3	46.4	+++	−	+++
Sphingomonas sp. strains						
F199	26.7	2.2	38.0	++	+	+++
B0477	17.7	2.1	42.3	++	+++	−
B0478	36.7	5.0	35.8	++	+++	−
B0522	39.5	3.9	28.4	++	+++	−
B0695	24.5	1.2	20.9	++	+	+++
B0712	38.8	3.0	31.3	++	+++	−

TABLE 4. Relative growth of subsurface and ATCC *Sphingomonas* strains on select aromatic compounds

Compound	Relative growth of[a]:							
	S. capsulata	*S. paucimobilis*	F199	B0477	B0478	B0522	B0695	B0712
Toluene	−	−	+	−	+	+	−	+
Naphthalene	−	−	+	−	+	+	−	−
p-Cresol	+	−	++	−	−	++	++	−
o-Xylene	−	−	+	−	+	−	+	+
m-Xylene	−	−	++	−	−	++	++	++
p-Xylene	−	−	++	−	++	−	−	−
Salicylate	+	+	++	+	++	++	++	++
Benzoate	−	−	+	+	+	+	+	+

[a] Symbols: −, no growth; + and ++, relative robustness of growth compared with that of a control consisting of cells plated onto mineral salt agar without a carbon source.

spp. There were some distinct differences, however, in the relative abundances of specific fatty acids in the two American Type Culture Collection (ATCC) strains, *S. capsulata* and *S. paucimobilis*, and the subsurface isolates. For example, the ATCC strains contained less 2-OH15:0 and more 2-OH14:0 (Table 3). There were also distinct differences in the relative abundance of three sphinganine bases. The base cy20:0 was present in all of the subsurface strains but absent in *S. capsulata* and *S. paucimobilis*, while cy21:0 was abundant in the ATCC strains, F199, and B0695 but absent in the other subsurface strains.

Growth on and mineralization of aromatic compounds. All of the subsurface strains with the exception of isolate B0477 were able to grow on a broad range of aromatic compounds (Table 4), although the patterns differed from that of strain F199. Strain B0477 grew only on salicylate and benzoate, while ATCC strains *S. capsulata* and *S. paucimobilis* grew on *p*-cresol and salicylate and on salicylate only, respectively. Subsurface strains F199, B0478, B0522, B0695, and B0712 were all able to clear fluorene, biphenyl, and dibenzothiophene on agar plates (Table 5), whereas B0477, *S. capsulata*, and *S. paucimobilis* were unable to do so. Orange metabolites were also produced by those bacteria that could clear dibenzothiophene plates. Mineralization of ^{14}C-labeled compounds was used as a quantitative measure of the ability of the *Sphingomonas* strains to degrade toluene and naphthalene. All of the subsurface isolates, again with the exception of B0477, mineralized between 6 and 17% of the [^{14}C]toluene and between 40 and 69% of the [^{14}C]naphthalene within a 48-h period (Fig. 2). Neither of the ATCC *Sphingomonas* strains nor subsurface isolate B0477 was able to mineralize these compounds.

Summary. The results of the 16S rRNA gene sequence and cellular fatty acid analyses clearly indicate that subsurface strains F199, B0477, B0478, B0522, B0695, and B0712 are *Sphingomonas* spp. Among those bacteria currently in the RDP, these strains are most closely related to *S. capsulata*. However, the subsurface isolates differ from *S. capsulata* in their 16S rRNA gene sequences and lipid compositions. The subsurface strains also differ from *S. capsulata* in their ability to degrade the broad range of aromatic compounds that was reported earlier for strain F199 (14). These results indicate that, while closely related to *S. capsulata*, the subsurface strains are clearly distinct, possibly representing new species. A more thorough characterization of the subsurface strains (now in progress) will be required to determine their specific taxonomic placement more accurately.

Although the ability to degrade toluene or naphthalene is relatively common among gram-negative aerobic heterotrophic bacteria, reports of single organisms able to degrade both of these compounds are rare (18). The finding of additional subsurface strains that can degrade this range of aromatics is significant in that bacteria with these abilities may be common throughout the Southeast Coastal Plain subsurface. Although two of the subsurface *Sphingomonas* strains came from the same sample (B0695 and B0712 came from a Middendorf Formation sample collected at a depth of 259 m at borehole P24 on DOE's Savannah River Site), most of the strains came from distinct samples and formations. F199 came from the Middendorf Formation, as did B0712 and B0695, but F199 was isolated from borehole C10, which was located 25 km from borehole P24. It is likely that additional *Sphingomonas* strains, including some with similar degradative properties, are among the several thousand strains from deep Southeast Coastal Plain sediments that are currently housed in the Subsurface Microbial Culture Collection.

F199 harbors two large plasmids (14), and approximately one in three aerobic heterotrophic bacteria isolated from deep Southeast Coastal Plain subsurface sediments harbors high-molecular-weight plasmids (15). It is not known whether the F199 plasmids harbor genes involved in the catabolism of one or more of the aromatic compounds or whether the other subsurface isolates that can degrade the same array of compounds as F199 contain plasmids. Since it is relatively common for the genes for aromatic catabolism to be encoded on trans-

TABLE 5. Ability of the subsurface and ATCC *Sphingomonas* strains to clear agar plates sprayed with ethereal solutions of fluorene, biphenyl, and dibenzothiophene

Compound	Clearing ability of[a]:							
	S. capsulata	*S. paucimobilis*	F199	B0477	B0478	B0522	B0695	B0712
Fluorene	−	−	+	−	+	+	+	+
Biphenyl	−	−	+	−	+	+	+	+
DBT[b]	−	−	+	−	+	+	+	+

[a] −, no clearing; +, significant zones of clearing around colonies.
[b] DBT, dibenzothiophene.

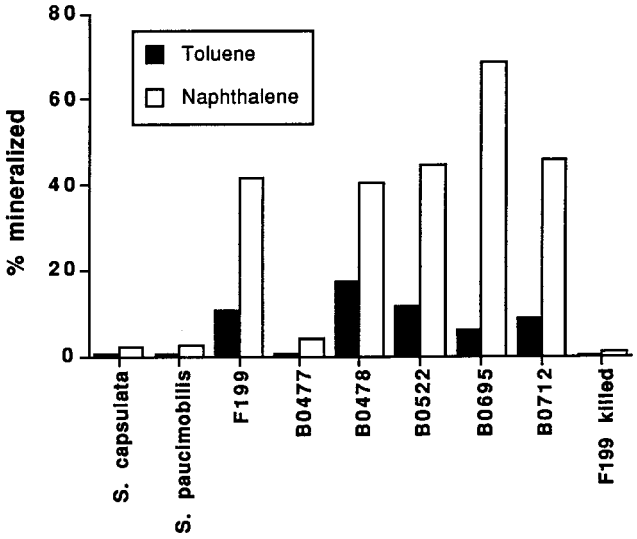

FIG. 2. Mineralization of [^{14}C]toluene and [^{14}C]naphthalene by the various *Sphingomonas* strains after incubation for 48 h.

missible plasmids, the possibility exists that the location of such genes on plasmids in subsurface *Sphingomonas* spp. may in part be responsible for their apparent broad dissemination in the subsurface. Whether the F199 plasmids harbor genes for aromatic catabolism and whether the other subsurface *Sphingomonas* strains harbor plasmids with similar genes are currently under investigation.

Sphingomonas spp. are relatively ubiquitous in soil, water, and sediments. As a group, *Sphingomonas* spp. have broad catabolic capabilities and therefore high potential for bioremediation and waste treatment. The range of contaminants that various *Sphingomonas* spp. (mainly *S. paucimobilis*) can degrade is extensive and includes dibenzo-*p*-dioxin and dibenzofuran (40), hexachlorocyclohexane (19), chlorinated biphenyls (37), halogenated diphenyl ethers (33), naphthalenesulfonic acids (23), toluene, naphthalene, and xylene (14), and polyaromatic hydrocarbons (28). This list is likely to increase as bacteria previously classified as *Pseudomonas* or *Flavobacterium* spp. are reclassified as *Sphingomonas* spp. *S. paucimobilis* (ATCC 29837) was previously called *Pseudomonas paucimobilis*, and *S. capsulata* (ATCC 14666) was previously called *Flavobacterium capsulatum*. Similarly, a pentachlorophenol-degrading bacterium previously described as a *Flavobacterium* sp. (35) is now believed to be a *Sphingomonas* sp. (8). In addition to their catabolic capabilities, *Sphingomonas* spp. are known to produce a number of different exopolysaccharides (31), some which may have commercial applications.

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