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Phylogeography of Sulfate-Reducing Bacteria among Disturbed Sediments, Disclosed by Analysis of the Dissimilatory Sulfite Reductase Genes (*dsrAB*)

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Sediment samples were collected worldwide from 16 locations on four continents (in New York, California, New Jersey, Virginia, Puerto Rico, Venezuela, Italy, Latvia, and South Korea) to assess the extent of the diversity and the distribution patterns of sulfate-reducing bacteria (SRB) in contaminated sediments. The SRB communities were examined by terminal restriction fragment (TRF) length polymorphism (TRFLP) analysis of the dissimilatory sulfite reductase genes (*dsrAB*) with NdeII digests. The fingerprints of *dsrAB* genes contained a total of 369 fluorescent TRFs, of which <20% were present in the GenBank database. The global sulfidogenic communities appeared to be significantly different among the anthropogenically impacted (petroleum-contaminated) sites, but nearly all were less diverse than pristine habitats, such as mangroves. A global SRB indicator species of petroleum pollution was not identified. However, several *dsrAB* gene sequences corresponding to hydrocarbon-degrading isolates or consortium members were detected in geographically widely separated polluted sites. Finally, a cluster analysis of the TRFLP fingerprints indicated that many SRB microbial communities were most similar on the basis of close geographic proximity (tens of kilometers). Yet, on larger scales (hundreds to thousands of kilometers) SRB communities could cluster with geographically widely separated sites and not necessarily with the site with the closest proximity. These data demonstrate that SRB populations do not adhere to a biogeographic distribution pattern similar to that of larger eukaryotic organisms, with the greatest species diversity radiating from the Indo-Pacific region. Rather, a patchy SRB distribution is encountered, implying an initially uniform SRB community that has differentiated over time.

Historically, a wide variety of chemical compounds have been discharged into bodies of water, generating serious environmental hazards. Many of these chemicals ultimately reside in sediments, where sequestration decreases their bioavailability for microbial transformation and anaerobic processes become the main route to remediation (11). In anoxic environments, such as estuarine sediments, sulfate-reducing bacteria (SRB) are major contributors to carbon and sulfur cycles (reviewed in references 25 and 40). SRB use sulfate as an electron acceptor while oxidizing diverse carbon sources, including petroleum hydrocarbon components (3, 17, 34, 35, 41). For many environments, characterization of SRB has been attempted by cultivation (29), phylogeny of 16S rRNA genes (30), or fluorescence in situ hybridization (31). However, these viable-count approaches or group-specific methods are limited in disclosing the in situ sulfidogenic communities by focusing mainly on members of the delta class of the phylum *Proteobacteria*.

Recently, a molecular approach based on the dissimilatory sulfite reductase genes (*dsrAB*) has been used to characterize SRB in a variety of environmental settings (5, 7, 8, 10, 13, 14, 19, 21–24, 27, 28, 35). Dissimilatory sulfite reductase catalyzes the last step in the sulfate reduction pathway and is unique to SRB (reviewed in references 35 and 40). In this study, *dsrAB* target genes were analyzed by terminal restriction fragment length polymorphism (TRFLP) analysis (4, 16) to assess the composition of sulfidogenic communities in a cosmopolitan collection of sediments. This analysis was conducted to elucidate how sulfidogenic communities are composed worldwide, to explore the hydrocarbon biodegradation potential globally, and to determine whether biogeographic patterns are apparent in SRB communities. Ultimately, the SRB population data may help in defining the resiliency of the ecosystem to anthropogenic impact and may provide molecular targets for determining the SRB responsible for biodegradation in situ.

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MATERIALS AND METHODS

Sample collection. Samples were collected from anthropogenically impacted sediments at 16 locations in the continental United States (in California, New Jersey, New York, and Virginia), South Korea, Italy, Latvia, Venezuela, and Puerto Rico (Table 1) representing freshwater and estuarine settings on four continents (Fig. 1). Multiple geographic samples were collected in Venice, Italy ($n = 4$); Venezuela ($n = 2$); Norfolk, Va. ($n = 2$); and different regions of New York State ($n = 2$). Most of the sites represent areas impacted by petroleum hydrocarbon components. Control unimpacted sites included pristine mangrove forests samples (Fajardo) from Puerto Rico and sediment from Mono Lake in California.

Community assessment based on TRFLP analysis. Total genomic DNA was extracted and purified from the sediments at each sampling site with subsamples (200 mg [wet weight]) coupled with multiple freeze-thaw and phenol-chloroform extraction as described previously (28). Primers DSR1F (5′-ACSCACTGGAAGCACG, labeled at the 5′ end with 6-carboxyfluorescein; Perkin-Elmer Life Sciences Inc., Boston, Mass.) and DSR4R (5′-GTGTAGCAGTTACCGCA) were used to amplify ~1.9 kb of the *dsrAB* gene. Each PCR contained 25 ng of

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TABLE 1. Description of sites studied and distribution of *dsr*-TRFs among the sediments studied

Site	Code	Quality	Habitat	Total no. of TRFs	Predicted no. of TRFs[a]	Accountability[b]
Arthur Kill, N.Y.	AK	Disturbed	Estuarine	78	30	38.5
Lielupe River, Latvia	Lt	Disturbed	Freshwater	39	10	25.6
Mono Lake, Calif.	ML	Undisturbed	Hypersaline	99	28	28.3
Norfolk, Va.	Nx	Disturbed	Estuarine	78	23	29.5
Onondaga Lake, N.Y.	OL	Disturbed	Freshwater	76	28	36.8
Puerto Rico	CR1r	Undisturbed	Mangrove	100	36	36.0
Puerto Rico	CR3b	Undisturbed	Mangrove	77	34	44.2
Puerto Rico	F2b	Undisturbed	Mangrove	92	32	34.8
Puerto Rico	F9r	Undisturbed	Mangrove	84	27	32.1
Puerto Rico	M2	Disturbed	Mangrove	46	18	39.1
Puerto Rico	P1	Disturbed	Mangrove	43	18	41.9
Shiwa Lake, South Korea	KS	Disturbed	Estuarine	84	30	35.7
Venezuela	Vcl	Disturbed	Estuarine	52	15	28.8
Venezuela	Vnal	Disturbed	Freshwater	98	38	38.8
Venice Lagoon, Italy	S1	Disturbed	Estuarine	119	31	26.1
Venice Lagoon, Italy	S3	Disturbed	Estuarine	114	33	28.9
Total				369	94	19.8

[a] TRF corresponding to a known *dsrAB* gene in GenBank (27).
[b] Percentage of the population (total TRFs) corresponding to known *dsr* genes.

template DNA and 20 pmol of primer, and the amplification conditions were 1 cycle of 94°C for 5 min, followed by 30 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1.5 min, with a final extension step of 72°C for 10 min. For each sediment sample, triplicate PCR amplifications of *dsrAB* genes were performed. After amplification, 20 ng of each *dsrAB* amplicon from the various sites was digested with NdeII (Roche Diagnostics Corporation, Indianapolis, Ind.) at 37°C for 2 h. The digested amplicons were precipitated with 35 μl of 95% ethanol and then centrifuged at 16,000 × *g* for 15 min. The DNA pellet was rinsed with 70% ethanol, dried, and resuspended in a mixture of 14.5 μl of deionized formamide and 0.5 μl of DNA fragment length internal standard (TAMRA 500; Perkin-Elmer Life Sciences Inc.). Fluorescently labeled TRFs were separated by capillary electrophoresis in an ABI 310 genetic analyzer. TRFLP information was analyzed with 310 Genescan version 3.1 software (Applied Biosystems Incorporated, Foster City, Calif.). The initial data analysis used a peak height detection of 25 fluorescence units for maximal TRF discovery prior to height normalization for sample comparison. Peaks were parsed to the nearest whole base pair and aligned by estimated size based on the TAMRA internal size standard. Differences in loading into the capillary for each sample were corrected by adjustment of all peak heights within a geographic site fingerprint to the average of triplicate TRFLPs for a single sample site. Specifically, the average of the total peak height for the entire fingerprint from replicates of a particular site (h_r) was used to normalize the total height of a particular sample (h_t). The resulting value (h_r/h_t) was applied to the height of each individual peak within a particular fingerprint for further data analysis. This corrected height was used for final peak detection

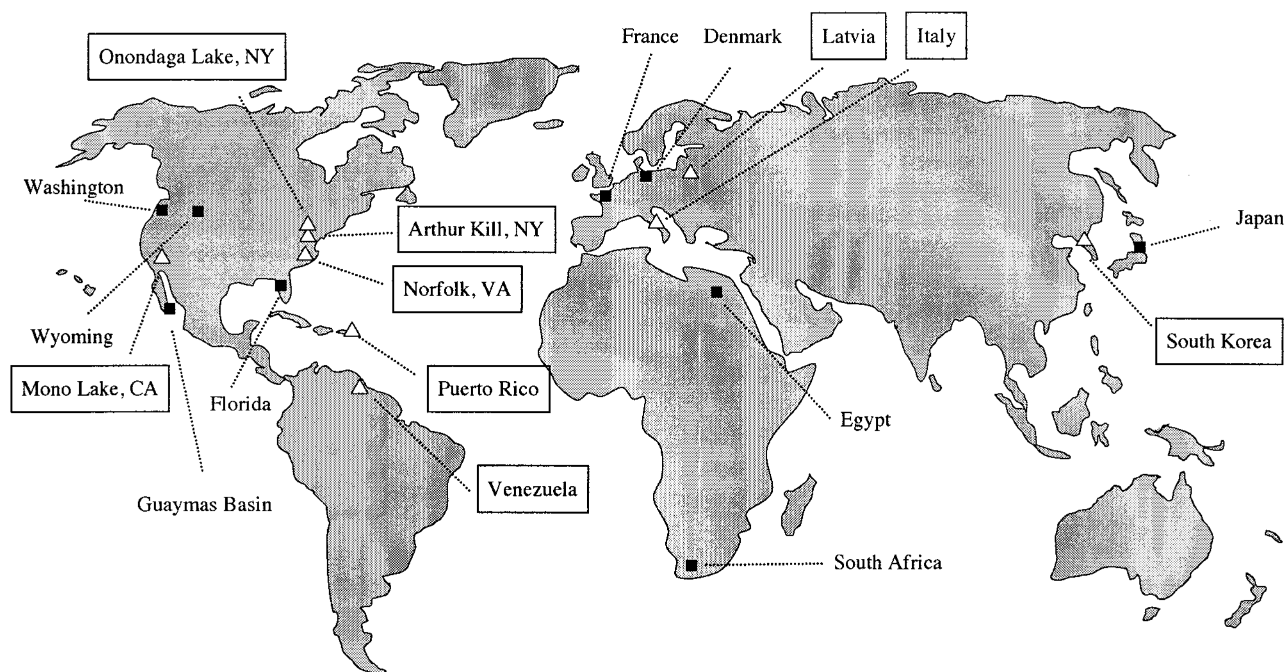

FIG. 1. Locations of sediments reporting *dsrAB* genes. Areas examined in this study (△) are boxed. The remaining sites described in the literature and used for in silico analyses are also shown (■).

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TABLE 2. In silico coverage of *dsrAB* genes in the GenBank database

Restriction enzyme (sequence)	No. of *dsr* genes in GenBank[a]			No. of diagnostic TRFs	Predicted percentage[c]	Highest TRF frequency[d]
	Cut	No cut	<50 bp[b]			
AluI (AG/CT)	225	61	95	56	59.1	92
Asp718 (G/GTACC)	12	294	75	7	3.1	65
BcnI (CCS/GG)	136	61	184	37	35.7	184
DraI (TTT/AAA)	15	354	12	8	3.9	12
HaeIII (GG/CC)	328	48	5	56	42.5	166
Hsp92II (CATG/)	360	12	9	61	73.2	81
NdeII (/GATC)	247	66	68	70	64.8	46
RsaI (GT/AC)	215	87	79	69	56.9	63
TaqI (T/CGA)	197	126	58	58	51.7	56

[a] Amount out of 381 sequences.
[b] TRF smaller than 50 bp.
[c] Percentage = 100 × (cut *dsr*/total *dsr*).
[d] Greatest number of taxons for a particular TRF.

with a value of 50 fluorescence units for determination of presence or absence to minimize detection threshold artifacts from the various geographic samples. Comparative analysis of TRFLP fingerprints was done on the basis of the Sørenson similarity index (18, 20) and the unweighted-pair group method using average linkages calculated with the COMbinatorial Polythetic Agglomerative Hierarchical clustering package (COMPAH96; http://www.es.umb.edu/faculty/edg/files/edgwebp.htm#COMPAH).

Sequencing, design of internal primers, and phylogenetic analysis of *dsrAB* genes. The *dsrAB* amplicons were cloned with the Topo TA cloning system (Invitrogen, Carlsbad, Calif.) in accordance with the manufacturer's instructions. Unique clones were screened and sequenced on an ABI 310 automated sequencer (ABI, Foster City, Calif.) with M13F/R, DSR1F, and the internal *dsrAB* sequencing primers PJdsr853R (5′-CGGTGMAGYTCGTCCTG) (28), PJdsr936F (5′-AGBBCRTAGCCRTGGACC), and PJdsr969R (5′-CATRTCG TCKYKCCAGGT). Sequences were compiled with Auto Assembler (ABI), Sequence Navigator (ABI), BLASTN (1), and the Fasta (26) software. Phylogenetic trees were reconstructed by Clustal X version 1.81 (36) and the neighbor-joining distance method with Jukes-Cantor correction (32). The bootstrap confidence levels were defined from 1,000 iterations of tree reconstruction (12).

Nucleotide sequence accession numbers. The GenBank accession numbers for the *dsrAB* genes described in this study are AY367723 through AY367740.

RESULTS

In order to assess the composition of sulfidogenic communities from the various geographic samples by TRFLP analysis of *dsrAB* genes (*dsr*-TRFLP), the resolving capability of various tetra-, penta-, and hexanucleotide restriction enzymes were tested in silico. However, many of the *dsrAB* sequences in the GenBank database were found to lack portions of the 5′ end. Therefore, prior to the in silico testing, missing bases were arbitrarily appended to the short *dsrAB* GenBank sequences with the sequence of *Desulfovibrio vulgaris* (accession no. U16723.1) (15) to provide a uniform starting point for TRFLP comparison. The number of *D. vulgaris* bases needed to standardize the various GenBank *dsrAB* entries was small (18 ± 5 bp) and did not likely contain a restriction site that would confound the in silico analysis.

Three hundred eighty-one *dsr* sequences were digested in silico with 37 different restriction enzymes. The results are shown in Table 2. All penta- and hexanucleotide restriction sites were not found to distinguish the various *dsrAB* genes from the GenBank database. For example, DraI (AAA/TTTA) could not resolve 354 *dsrAB* genes or 93% of the database. Many of the 4-bp cutters produced a larger number of TRFs that could identify more than 40% of the *dsrAB* genes in the database. However, most of these enzymes provided low resolution for the entire database. Specifically, the GGCC site, represented by HaeIII, produces the largest amount of TRFs, but the labeled TRF at bp 59 occurs 166 times or in 43.6% of the *dsrAB* sequences in the database. The restriction enzyme profile with the largest number of unique TRFs (best resolution) was NdeII (/GATC), which generated 70 TRFs. The various *dsrAB* genes from the GenBank database with their predicted NdeII TRF peaks can be found in reference 27.

Therefore, *dsr*-TRFLP analyses with NdeII were conducted to examine the heterogeneity of sulfidogenic communities in sediments worldwide. This fingerprinting generated 369 total peaks for all geographic samples (selected fingerprints are shown in Fig. 2). Two sites in the Venice Lagoon (Italy) exhibited the most diverse sulfidogenic communities, with 119 and 114 TRFs, followed by a pristine mangrove in Puerto Rico, Mono Lake (California), and a river in Venezuela with 100, 99, and 70 TRFs, respectively (Table 1). Roughly 4 to 10 TRFs in each fingerprint were found to have a relatively large fluorescent area (Fig. 2). Conversely, most of the *dsr*-TRFLP peaks represented less than 1% of the normalized total height of the fingerprint.

A majority of TRFs were found in the western hemisphere (73.8%) samples and in regions with a temperate climate (51.3%), compared with the eastern hemisphere (26.2%) samples and more tropical climates (48.7%). Twenty-one TRFs were found to have a cosmopolitan distribution, being found on all four continents (North America, South America, Asia, and Europe) and in the Caribbean. The various sizes of the cosmopolitan TRFs are 52, 53, 55, 60, 63, 64, 73, 97, 108, 141, 160, 164, 169, 170, 174, 192, 200, 211, 216, and 302 bp. However, some of these TRFs correspond to peaks with multiple representatives in the GenBank database (53, 69, 160, and 164 bp [27]) and may not truly be globally distributed. The Venice Lagoon (Italy), the Norfolk area (Virginia), and the mangrove site (Puerto Rico) contained the largest number of TRF peaks occurring a single time within the data set. The total number of TRF peaks and the percentage of peaks that can be identified with *dsrAB* sequences are shown in Table 1. Ninety-four of a possible 369 TRFs can be associated with *dsrAB* genes in the GenBank database (accountability), indicating that <20% of

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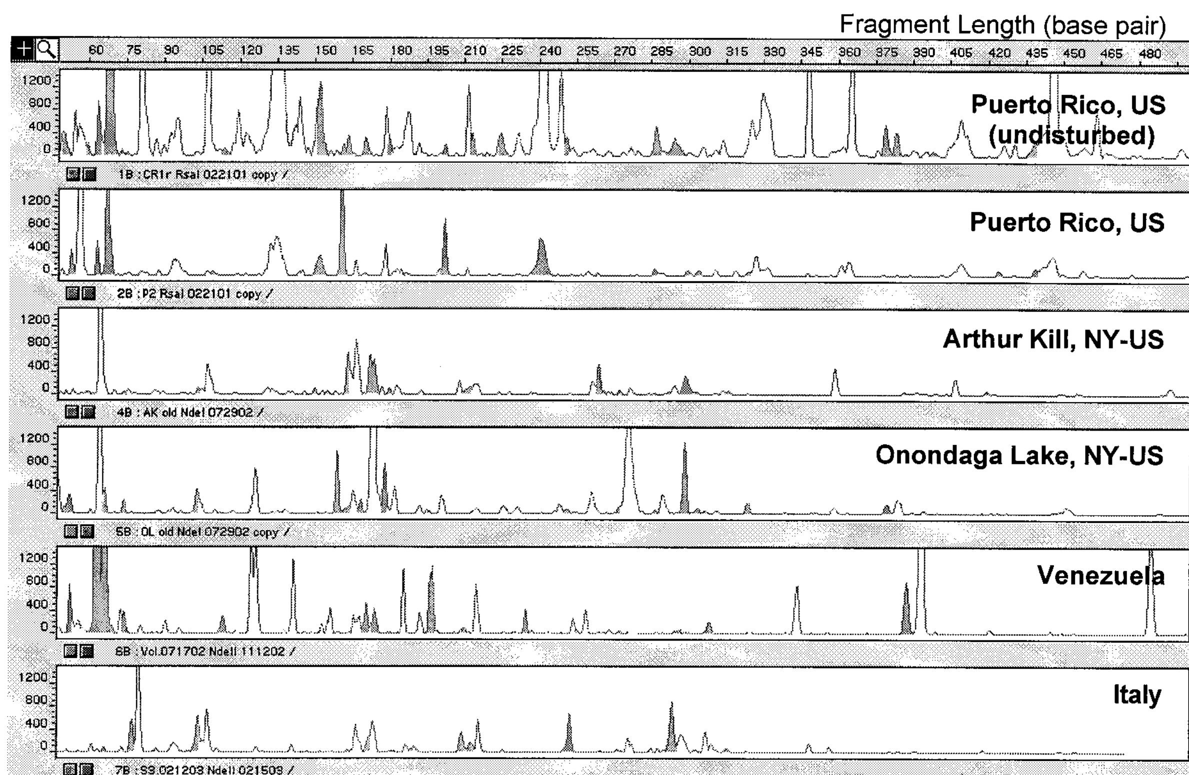

FIG. 2. Selected *dsr*-TRFLP fingerprints of disturbed and pristine sediments from around the world that were digested with NdeII. Highlighted peaks are represented in the GenBank database.

the *dsrAB* genes from the worldwide SRB community currently reside in the GenBank database.

To assess whether sulfidogenic communities from polluted sites were more similar to each other compared with pristine sites and whether geographic proximity was a major driver in structuring SRB populations, the *dsr*-TRFLP profiles from different locations were arranged with the Sørenson similarity index (C_s) and the unweighted-pair group method using average linkages (Fig. 3). Overall tree topology indicated that the samples from Puerto Rico, Venice, and Norfolk, Va., formed distinctive groups. For

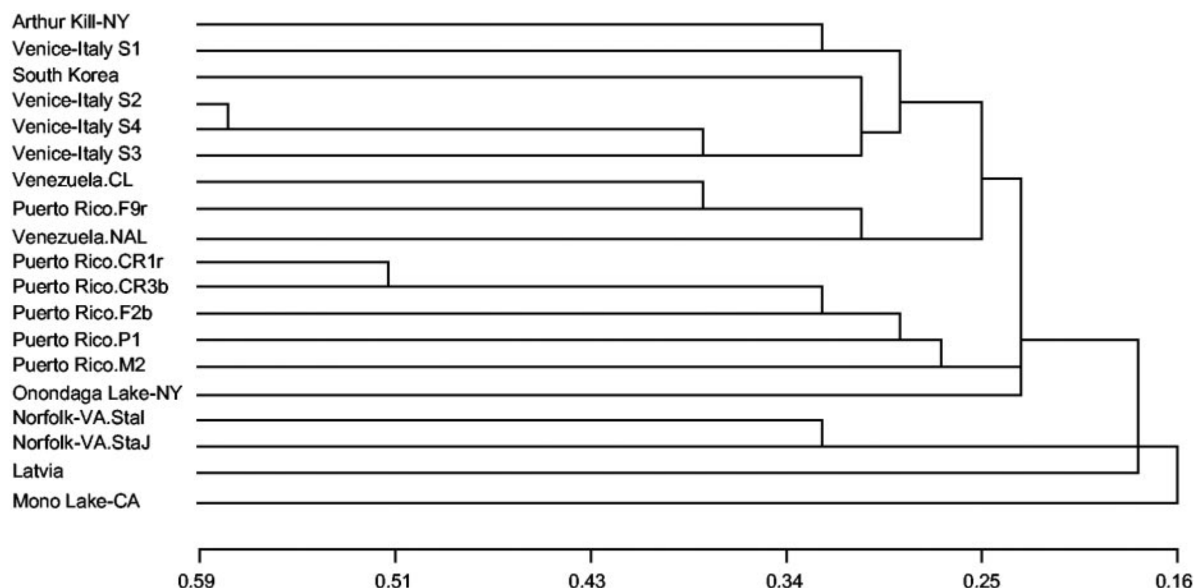

FIG. 3. Cluster analysis for sulfidogenic communities based on *dsr*-TRFLP and digestion with NdeII. The dendrogram was constructed with the Combinatorial Polythetic Agglomerative Hierarchical clustering software (COMPAH96).

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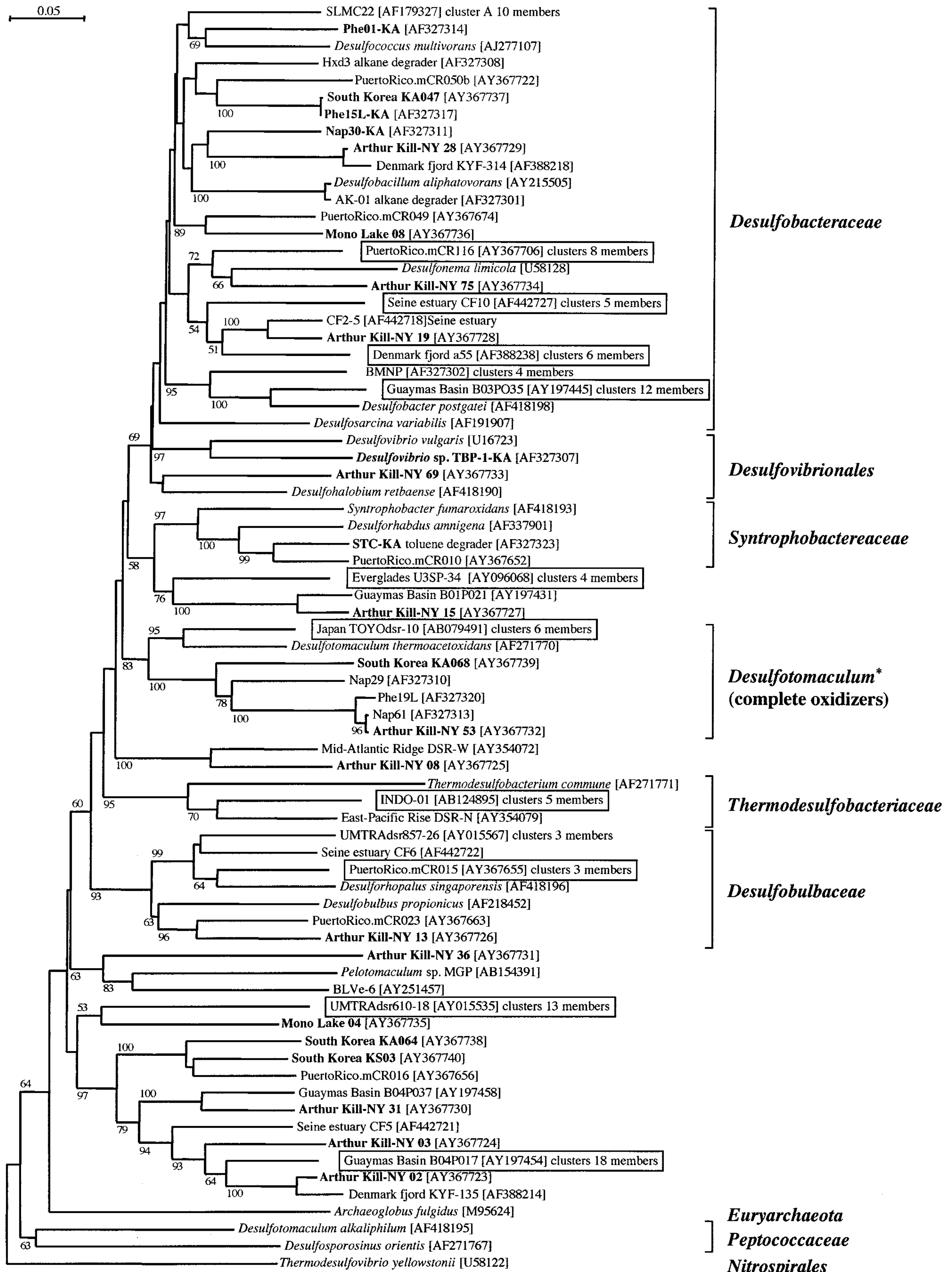

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example, the impacted sites (Ponce and Lajas) clustered with the pristine sites from Puerto Rico (Cabo Rojo and Fajardo) rather than with other impacted sites such as Arthur Kill or Norfolk. There did not appear to be a coherent pattern observable on the basis of freshwater, estuarine, or marine environments, suggesting that salinity was not a major driver for SRB communities. In contrast, the fingerprints of other samples from within the United States (Arthur Kill and Onondaga Lake), a riverine setting in South Korea, and the Latvian/Mono Lake, Calif., clustered with those of samples from geographically widely separated sites (Venezuela, Venice, and Puerto Rico).

Clonal libraries were established from Arthur Kill, South Korea, and Mono Lake sediments to identify some of the *dsrAB* genes not represented in the GenBank database. Preliminary screening has resulted in a diverse group comprising 18 novel *dsr* sequences and the retrieval of five known *dsrAB* genes associated with sulfidogenic consortia and strains of SRB capable of anaerobic degradation of hydrocarbons. For example, *dsr* sequences for *Desulfovibrio* sp. strain TBP-1 (a tribromophenol-degrading isolate from Arthur Kill, N.Y.), the toluene degrader STC (Raritan River, N.J.), and three clones from a polycyclic aromatic hydrocarbon-degrading consortium (Nap30, Phe01, and Phe15L from Arthur Kill) were detected in the Anyang river in South Korea (KA).

A *dsrAB* gene phylogenetic tree was reconstructed by neighbor-joining distance methods with 502 taxa and 350 bp of unambiguously aligned sequence to ascertain whether the genes form biogeographic clades (Fig. 4). The tree has been pared for clarity with the members of the many microclusters from the literature. The environmental *dsrAB* genes from this study were found to form deeply branched groups with relatives obtained from a fjord in Denmark, a neotropical manglal, and a French estuary, among others. Each environmental site studied so far has produced a diverse set of *dsrAB* genes mostly distributed in several endemic clusters. These microclusters are composed, for example, of 18 clones from the Guaymas basin (10), 13 from uranium mills (8), 10 from microbial mat (19), 8 from Puerto Rican manglal (27), 6 from Denmark fjords (37), 6 from Japanese metal mines (24), and 5 from the Central Indian Ridge (23). Roughly 70% of the environmental *dsrAB* genes form these endemic microgroups. The overall phylogeography of *dsrAB* genes suggests a high level of endemicity. The endemic microclusters are indicated by boxes in Fig. 4. However, there are instances in which a presumed endemic clade is no longer supported by bootstrap analysis with close representatives from geographically widely separated regions.

DISCUSSION

We examined *dsrAB* genes as a molecular marker in polluted and pristine sediments worldwide to elucidate the community composition of SRB, to establish whether reference biodegrading bacteria (28) are distributed worldwide, and to ascertain whether biogeographic patterns could be seen. Some major findings were that <20% of the *dsrAB* genes detected in the TRFLP fingerprint could be assigned to genes in the GenBank database. This number may be an underestimate since NdeII does not resolve many of the known *dsrAB* genes (Table 2) and a combination of different restriction enzymes will ultimately provide better resolution with TRFLP methodology. The fingerprints indicate that SRB communities under chronic anthropogenic impact (e.g., the Lajas, Ponce, and Venezuela collecting lagoons) contain roughly half of the SRB populations found in more pristine areas (natural reserves in Puerto Rico and Venezuela).

No TRF has been found to be common to all of the sites studied, implying that the notion of a universal SRB indicator for pollution or biodegradation is not likely. However, two biodegrading clones, STC and BMNP, associated with anaerobic toluene and benzene degradation (6, 28, 29) seem to be widely distributed. For example, the toluene-degrading bacterium (STC) originally isolated from the Raritan River in New Jersey (29) has been found in Arthur Kill and Latvia TRFLP fingerprints with the enzyme RsaI (27). Furthermore, a common *dsrAB* gene for a benzene-, methylnaphthalene-, and phenanthrene-degrading consortium (BMNP) (21) derived from both Atlantic and Pacific samples is detected with NdeII as part of a multitaxon TRF for many of the geographic sites reported here. This BMNP TRF was observed in the United States (Norfolk and Mono Lake), Italy, Venezuela, South Korea, and Puerto Rico (Fajardo and Ponce). Signature TRFs for other pollutant-degrading bacteria (21) such as alkane-degrading strain AK-01, dehalogenating *Desulfovibrio* sp. strain TBP-1, and the benzene-degrading consortium were also observed in Onondaga Lake, N.Y.; Venice Lagoon, Italy; and Puerto Rico (sample P1), respectively. Unfortunately, the identification of these pollutant-degrading bacteria was not definitive because the TRF for these *dsrAB* genes is shared with other *dsrAB* genes in the database (27). For example, the four members of the benzene-degrading consortium and clones from the naphthalene- and phenanthrene-degrading consortium (Nap 30, Phe15L, and Phe16L) produced a TRF of 69 bp. However, clonal libraries were found to contain *dsrAB* sequences for *Desulfovibrio* sp. strain TBP-1, STC, Nap30, Phe01, and Phe15L in the Anyang River (South Korea). These results imply a nearly worldwide potential for mineralizing petroleum hydrocarbons by specific microorganisms. While it is generally believed that hydrocarbon biodegradative potential exists around the globe, this is the first direct evidence that specific microorganisms (which are associated with hydrocarbon degradation) are present in samples from widely separated sites.

Finally, a biogeographic signature of SRB populations can

FIG. 4. Neighbor-joining phylogenetic tree of *dsrAB* genes from sediments (350-bp alignment). The prefix PuertoRico.mCR corresponds to a clone library established with a sediment sample from manglal in Cabo Rojo, Puerto Rico. The suffix KA indicates *dsrAB* sequences from hydrocarbon-degrading consortia or isolates that were also retrieved from the Anyang River in South Korea. Phylotypes within boxes represent endemic clusters from the site studied. The values at the nodes of branches are bootstrap percentages on 1,000 replicates. Bootstrap values higher than 50% are shown. The scale bar represents a 5% estimated change.

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be observed in our data. Biological sulfate reduction is considered an ancient process (2, 33, 35, 38), existing prior to the current continental configuration. Globally, sulfidogenic communities may have been distributed by four mechanisms analogous to the evolution of larger organisms (macrobes): homogeneous distribution, radiation from the Indo-Pacific region, restriction by major environmental conditions, or patchiness that has developed over a homogeneous background. We can assess those possibilities on the basis of pairwise comparisons of the Sørenson similarity index for *dsr*-TRFLP of sulfidogenic communities (Fig. 3). Primarily, a homogeneous distribution (everything is everywhere) is not considered likely since the *dsr*-TRFLP analysis demonstrated different SRB communities at the various geographic locations. However, there may be many *dsrAB* genes that are below the limit of detection by PCR and/or our restriction enzyme resolution that are not represented in the fingerprints. Second, a hypothetical diversification of SRB from the Indo-Pacific region is not supported since samples from sites such as South Korea did not cluster with samples from sites in close geographic proximity such as North American populations. The third possibility, restriction by easily measured environmental conditions (e.g., temperature), is supported by the clustering of various temperate groups (New York, Venice, and South Korea or Norfolk, Latvia, and California) and the tropical groups (Puerto Rico and Venezuela). Finally, the patchy distribution of SRB communities over a background is supported by the clustering of samples from Venice, Norfolk, and Puerto Rico. These molecular, non-culture-based results imply that many sediment-associated, sulfidogenic communities have emerged from SRB groups physically separated over geological time and have become adapted to local environmental conditions by genetic diversification, as is observed in culture-based studies with fluorescent *Pseudomonas* (9) and *Sulfolobus* (39) isolates from terrestrial and deep sea vent environments, respectively.

In conclusion, while disclosing the structure of sulfidogenic communities among disturbed sites, we provide insights about the diversity, uncharacterized extent, and biogeography of SRB. Our scrutiny of the *dsrAB* genes in a cosmopolitan collection of sediments indicates the presence of extremely diverse sulfidogenic communities that exhibit geographic patchiness. We do not really know how many different SRB species occupy a sample. Nor do we know how widely distributed are the SRB that have been shown to degrade petroleum hydrocarbons. Although it is believed that the potential for anaerobic hydrocarbon degradation is broadly dispersed, this study provides direct evidence of anaerobic hydrocarbon-degrading molecular markers detectable in samples from around the globe. Several *dsrAB* genes corresponding to hydrocarbon-degrading isolates or consortium members were detected in the TRFLP profiles and/or retrieved in the clonal libraries. Studies such as the one described here can form the basis of an improved understanding of the patterns of bacterial communities. This geographic information about SRB may provide clues to the mechanisms that control microbial diversity and drive community composition.

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REFERENCES

1. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. L. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. **Anbar, A. D., and A. H. Knoll.** 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? Science **297:**1137–1142.
3. **Annweiler, E., W., Michaelis, and R. U. Meckenstock.** 2001. Anaerobic cometabolic conversion of benzothiophene by a sulfate-reducing enrichment culture and in a tar-oil-contaminated aquifer. Appl. Environ. Microbiol. **67**:5077–5083.
4. **Avaniss-Aghajani, E., K. Jones, D. Chapman, and C. Brunk.** 1994. A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. BioTechniques **17:**144–146.
5. **Baker, B. J., D. P. Moser, B. J. MacGregor, S. Fishbain, M. Wagner, N. K. Fry, B. Jackson, N. Speolstra, S. Loos, K. Takai, B. S. Lollar, J. Fredrickson, D. Balkwill, T. C. Onstott, C. F. Wimpee, and D. A. Stahl.** 2003. Related assemblages of sulphate-reducing bacteria associated with ultradeep gold mines of South Africa and deep basalt aquifers of Washington State. Environ. Microbiol. **5:**267–277.
6. **Beller, H. R., A. M. Spormann, P. K. Sharma, J. R. Cole, and M. Reinhard.** 1996. Isolation and characterization of a novel toluene-degrading, sulfate-reducing bacterium. Appl. Environ. Microbiol. **62:**1188–1196.
7. **Castro, H., K. R. Reddy, and A. Ogram.** 2002. Composition and function of sulfate-reducing prokaryotes in eutrophic and pristine areas of the Florida Everglades. Appl. Environ. Microbiol. **68:**6129–6137.
8. **Chang, Y. J., A. D. Peacock, P. E., Long, J. R., Stephen, J. P. McKinley, S. J., Macnaughton, A. K. Hussain, A. M. Saxton, and D. C. White.** 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. Appl. Environ. Microbiol. **67:**3149–3160.
9. **Cho, J. C., and J. M. Tiedje.** 2000. Biogeography and degree of endemicity of fluorescent *Pseudomonas* strains in soil. Appl. Environ. Microbiol. **66:** 5448–5456.
10. **Dhillon, A., A. Teske, J. Dilon, D. A. Stahl, and M. L. Sogin.** 2003. Molecular characterization of sulfate-reducing bacteria in the Guaymas Basin. Appl. Environ. Microbiol. **69:**2765–2772.
11. **Eggleton, J., and K. V. Thomas.** 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int. **30:**973–980.
12. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**166–170.
13. **Fishbain, S., Dillon, J. G., Gough, H. L., and D. A. Stahl.** 2003. Linkage of high rates of sulfate reduction in Yellowstone hot springs to unique sequence types in the dissimilatory sulfate respiration pathway. Appl. Environ. Microbiol. **69:**3663–3667.
14. **Fukuba, T., M. Ogawa, T. Fujii, and T. Naganuma.** 2003. Phylogenetic diversity of dissimilatory sulfite reductase genes from deep-sea cold seep sediment. Mar. Biotechnol. **5:**458–468.
15. **Karkhoff-Schweizer, R. R., D. P. Huber, and G. Voordouw.** 1995. Conservation of the genes for dissimilatory sulfite reductase from *Desulfovibrio vulgaris* and *Archaeoglobus fulgidus* allows their detection by PCR. Appl. Environ. Microbiol. **61:**290–296.
16. **Kitts, C. L.** 2001. Terminal restriction fragment patterns: a tool for comparing microbial communities and assessing community dynamics. Curr. Issues Intest. Microbiol. **2:**17–25.
17. **Kleikemper, J., M. H. Schroth, W. V. Sigler, M. Schmucki, S. M. Bernasconi, and J. Zeyer.** 2003. Activity and diversity of sulfate-reducing bacteria in a petroleum hydrocarbon-contaminated aquifer. Appl. Environ. Microbiol. **68:** 1516–1523.
18. **Magurran, A. E.** 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N.J.
19. **Minz, D., J. L. Flax, S. J. Green, G. Muyzer, Y. Cohen, M. Wagner, B. E. Rittmann, and D. A. Stahl.** 1999. Diversity of sulfate-reducing bacteria in oxic and anoxic regions of a microbial mat characterized by comparative analysis of dissimilatory sulfite reductase genes. Appl. Environ. Microbiol. **65:**4666–4671.
20. **Murray, A. E., C. M. Preston, R. Massana, L. T. Taylor, A. Blakis, K. Wu, and E. F. DeLong.** 1998. Seasonal and spatial variability of bacterial and

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archeal assemblages in the coastal waters near Anvers Island, Antarctica. Appl. Environ. Microbiol. **64:**2585–2595.

21. **Nakagawa, T., and M. Fukui.** 2003. Molecular characterization of community structures and sulfur metabolism within microbial streamers in Japanese hot springs. Appl. Environ. Microbiol. **69:**7044–7057.
22. **Nakagawa, T., J. Ishibashi, A. Maruyama, T. Yamanaka, Y. Morimoto, H. Kimura, T. Urabe, and M. Fukui.** 2004. Analysis of dissimilatory sulfite reductase and 16S rRNA gene fragments from deep-sea hydrothermal sites of the Suiyo Seamount, Izu-Bonin Arc, Western Pacific. Appl. Environ. Microbiol. **70:**393–403.
23. **Nakagawa T., S. Nakagawa, F. Inagaki, K., Takai, and K. Horikoshi.** 2004. Phylogenetic diversity of sulfate-reducing prokaryotes in active deep-sea hydrothermal vent chimney structures. FEMS Microbiol. Lett. **232**:145–152.
24. **Nakagawa, T., S. Hanada, A. Maruyama, K. Marumo, T. Urabe, and M. Fukui.** 2002. Distribution and diversity of thermophilic sulfate-reducing bacteria within a Cu-Pb-Zn mine (Toyoha, Japan). FEMS Microbiol. Ecol. **1378:**1–11.
25. **Nealson, K. H.** 1997. Sediment bacteria: who's there, what are they doing, and what's new? Annu. Rev. Earth Planet. Sci. **25:**403–434.
26. **Pearson, W. R., and D. L. Lipman.** 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA **85:**2444–2448.
27. **Pérez-Jiménez, J. R.** 2003. Molecular characterization of sulfidogenic communities among pristine and polluted sites based on the dissimilatory sulfite reductase genes (*dsrAB*). Ph.D. thesis. Rutgers University, New Brunswick, N.J.
28. **Pérez-Jiménez, J. R., L. Y. Young, and L. J. Kerkhof.** 2001. Molecular characterization of sulfate-reducing bacteria in anaerobic hydrocarbon-degrading consortia and pure cultures using the dissimilatory sulfite reductase (*dsrAB*) genes. FEMS Microbiol. Ecol. **35:**145–150.
29. **Phelps, C. D.** 1998. Anaerobic biodegradation of aromatic petroleum hydrocarbons. Ph.D. thesis. Rutgers University, New Brunswick, N.J.
30. **Phelps, C., L. J. Kerkhof, and L. Y. Young.** 1998. Molecular characterization of a sulfate-reducing consortium which mineralizes benzene. FEMS Microbiol. Ecol. **27:**269–279.
31. **Purdy, K. J., T. M. Embley, and D. B. Nedwell.** 2002. The distribution and activity of sulphate reducing bacteria in estuarine and coastal marine sediments. Antonie van Leeuwenhoek **81:**181–187.
32. **Saitou, N., and M. Nei.** 1987. The neighbor joining method: a new method for constructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
33. **Shen, Y., R. Buick, and D. E. Canfield.** 2001. Isotopic evidence for microbial sulphate reduction in the early Archaean era. Nature **410:**77–81.
34. **So, C. M., and L. Y. Young.** 1999. Isolation and characterization of a sulfate-reducing bacterium that anaerobically degrades alkanes. Appl. Environ. Microbiol. **65:**2969–2976.
35. **Stahl, D. A., S. Fishbain, M. Klein, B. J. Baker, and M. Wagner.** 2002. Origins and diversification of sulfate-respiring microorganisms. Antonie Van Leeuwenhoek **81:**189–195.
36. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.** 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **24:**4876–4882.
37. **Thomsen, T. R., K. Finster, and N. B. Ramsing.** 2001. Biogeochemical and molecular signatures of anaerobic methane oxidation in a marine sediment. Appl. Environ. Microbiol. **67:**1646–1656.
38. **Wagner, M., A. J. Roger, L. Flax, G. A. Brusseau, and D. A. Stahl.** 1998. Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J. Bacteriol. **180:**2975–2982.
39. **Whitaker, R. J., D. W. Grogan, and J. W. Taylor.** 2003. Geographic barriers isolate endemic populations of hyperthermophilic Archaea. Science **301:**976–978.
40. **Widdel, F., and T. A. Hansen.** 1999. The dissimilatory sulfate- and sulfur-reducing bacteria. *In* M. Dworkin, K.-H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes: an evolving electronic database for the microbiological community, 3rd ed., release 3.0. [Online.]. Springer-Verlag, New York, N.Y. http://link.springer-ny.com/link/service/books/10125.
41. **Zhang, X., and L. Y. Young.** 1997. Carboxylation as an initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. Appl. Environ. Microbiol. **63:**4759–4764.

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