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ORIGINAL PAPER

Characterization of the indigenous PAH-degrading bacteria of *Spartina* dominated salt marshes in the New York/New Jersey Harbor

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Abstract The aerobic polyaromatic hydrocarbon (PAH) degrading microbial communities of two petroleum-impacted *Spartina*-dominated salt marshes in the New York/New Jersey Harbor were examined using a combination of microbiological, molecular and chemical techniques. Microbial isolation studies resulted in the identification of 48 aromatic hydrocarbon-degrading bacterial strains from both vegetated and non-vegetated marsh sediments. The majority of the isolates were from the genera *Paenibacillus* and *Pseudomonas*. Radiotracer studies using ^{14}C-phenanthrene and ^{14}C-pyrene were used to measure the PAH-mineralization activity in salt marsh sediments. The results suggested a trend towards increased PAH mineralization in vegetated sediments relative to non-vegetated sediments. This trend was supported by the enumeration of PAH-degrading bacteria in non-vegetated and vegetated sediment using a Most Probable Numbers (MPN) technique, which demonstrated that PAH-degrading bacteria existed in non-vegetated and vegetated sediments at levels ranging from 10^2 to 10^5 cells/g sediment respectively. No difference between microbial communities present in vegetated versus non-vegetated sediments was found using terminal restriction fragment length polymorphism (of the 16S rRNA gene) or phospholipid fatty acid analysis. These studies provide information on the specific members and activity of the PAH-degrading aerobic bacterial communities present in *Spartina*-dominated salt marshes in the New York/New Jersey Harbor estuary.

Keywords *Paenibacillus* · Polyaromatic hydrocarbons · Marsh sediment · *Spartina*

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Introduction

Salt marshes are some of the most productive ecosystems on earth, promoting enhanced plant and microbial activity, as well as providing important habitat for fish, bird and other wildlife (Mitsch and Gosselink 2000). Salt marshes are key elements to the ecology of the New York/New Jersey (NY/NJ) Harbor estuary. Recent studies have indicated that the environmental ecological conditions in the Harbor are poor (US Environmental Protection Agency 2003; Steinberg et al. 2004). Sediment contamination, loss of wetlands, and poor fish and benthos

condition are all major concerns in the NY/NJ Harbor estuary (Steinberg et al. 2004).

Toxic contaminants that are of concern in the NY/NJ Harbor estuary include polyaromatic hydrocarbons (PAHs) which enter the harbor through accidental release of petroleum products, by deposition of airborne particles, and from combined sewer overflows and storm drains (Huntley et al. 1995; Jones et al. 1998; Gigliotti et al. 2002; Steinberg et al. 2004). It is estimated that over one hundred billion liters of petroleum and petroleum products are transported yearly through the NY/NJ Harbor and over 67 million liters of these petroleum products were spilled into the Newark Bay area alone between 1986 and 1991 (Jones et al. 1998). Petroleum influx of this volume, in conjunction with input from other sources has lead to the continued accumulation of PAHs in the sediments of the NY/NJ Harbor, with sediment concentrations at some locations as high as 59 mg/kg (total PAHs) (Jones et al. 1998). PAHs in harbor sediments include many highly carcinogenic PAHs (Jones et al. 1998) and suspended harbor sediments have been demonstrated to adversely affect harbor biota (Litten 2003).

PAHs accumulate and persist in sediments because of their chemical and biological stability (Keck et al. 1989; Van Metre and Mahler 2005). Remedies for PAH-contaminated sediment are sought in the NY/NJ Harbor region and elsewhere. Because of the sensitive nature of salt marshes, degradation of petroleum hydrocarbons by existing microbial communities (natural attenuation) is considered the remediation method of choice (Zhu et al. 2004).

Laboratory and field trials demonstrating microbial degradation of petroleum hydrocarbons (including PAHs) in salt marshes have mainly focused on the influence of temperature, nutrients, oxygen, pH and salinity (reviewed in Zhu et al. 2004). Only a very few studies have compared microbial petroleum hydrocarbon degradation in vegetated versus non-vegetated marine and salt marsh sediments in the lab (Wright et al. 1997, 2004; Launen et al. 2002) or the field (Garcia-Blanco et al. 2007) and with the exception of the studies conducted by Daane et al. (2001, 2002) none have characterized the specific PAH-degrading microbial communities present in *Spartina*-dominated petroleum impacted salt marshes.

The objective of the present study was to characterize the indigenous PAH-degrading bacteria occurring in petroleum-impacted *Spartina*-dominated salt marshes in the New York/New Jersey Harbor estuary. This was accomplished using a combination of pure culture and mesocosm-based microbiological, molecular and chemical techniques.

Materials and methods

Sampling locations

Sediment samples were collected from vegetated and non-vegetated areas at two separate salt marsh sites: the Gulfport Reach site and a petroleum refinery site, both within the NY/NJ Harbor estuary.

The Gulfport Reach is located on the Arthur Kill, a tidal strait between Staten Island and New Jersey that is heavily trafficked with oil barges and tankers. Repeated spill events have generated a substantial amount of petroleum contamination; for example, in 1990 2.5 million liters of no. 2 fuel oil spilled from an underwater pipe rupture, which destroyed 8 ha of *S. alterniflora* in the immediate region (Bergen et al. 2000). Re-planting of *S. alterniflora* into much of the oiled sediments of the Gulfport Reach was conducted in 1991, however despite success in other locations affected by the 1991 spill, predation from Canada geese and damage from wave action in the Kill prevented the re-establishment of *S. alterniflora* in parts of the de-vegetated zone of the Gulfport Reach (from mean low tide level up to 1.25 m above the high tide level). At the last monitoring time (1993) the mean TPH in the Gulfport Reach sediment was 7,538 μg/g (0.7% wt/wt), with TPH levels at some locations as high as 16% (Bergen et al. 2000).

The second sampling site was a salt marsh located at an operating oil refinery site (Refinery Site). The site has been used as an oil refinery for almost 100 years, with at least 20 years of historical petroleum sludge deposition occurring in the sampling area. Petroleum residue was present at high levels and had seeped to the surface of the sediments. Total petroleum hydrocarbons (TPH) and PAH levels at the site ranged from 0.5 to 25% (wt/wt), and PAH levels ranged from 5 to 100 mg/Kg (E. Drake, personal communication). The area was vegetated with *S. alerniflora*, *S. patens* and *Phragmites australis* around the periphery of the most contaminated zone. The TPH concentrations in the non-vegetated area were typically 6–9% (wt/wt), while those in the

vegetated areas were reduced to 1.5–4.0% (wt/wt). Vegetation did not grow in the most heavily contaminated areas of this site (E. Drake, personal communication).

Sample collection

The Gulfport Reach site was sampled during July of 1999 and May, June and November of 2000 with the assistance of M. Levandowsky (Pace University) and C. Alderson (New York City Department of Parks & Recreation). At all sampling times samples were collected along a tidal transect, from the low to high tide points (sampling points labeled A through E: see Fig. 1). Point A was in an area where *S. alterniflora* is unlikely to grow due to water level and wave action, B was in the de-vegetated zone where *S. alterniflora* grew before the 1991 spill but had not re-established despite replanting. Point C was at the fringe of the de-vegetated zone and the beginning of *S. alterniflora* cover, D and E were within heavily vegetated areas where *S. alterniflora* was not impacted by the 1991 spill. The date and purpose of each sampling conducted at the Gulfport Reach site was as follows: Samples for bacterial isolation were collected at the Gulfport Reach in June of 1999 for bacterial isolation only. Samples for ^{14}C-PAH degradation and MPN studies were collected in June of 2000. Samples for FAME analysis were collected in May, June and November of 2000. Samples for TRFLP analysis were collected during May and June of 2000.

At the refinery site samples were collected from tidally influenced areas vegetated with either *S. alterniflora* or *S. patens* (see Fig. 1). Only vegetated sediment samples were collected from the refinery site. The date and purpose of each sampling conducted at the refinery site was as follows: Samples for bacterial isolation were collected during July of 1999. Samples for ^{14}C-PAH degradation and MPN studies were collected in June of 2000.

All samples were collected from the top 15 cm (vertical distance) of sediment. At each location sampled for ^{14}C-PAH degradation, MPN, FAME and TRFLP analysis three replicate samples were collected, each approximately 10 cm (horizontal distance) distant from the other (see Fig. 1). Samples collected for bacterial isolation were not replicated as the bacterial isolation studies are not quantitative in nature. Vegetated samples included plant root material and adjacent sediment. Samples were collected using garden trowels and spades that were surface-sterilized with 70% ethanol. Samples were collected into 50 ml sterile Falcon tubes. After collection, samples were transported to the laboratory within 4 h. Samples used for FAME or tRFLP analysis were

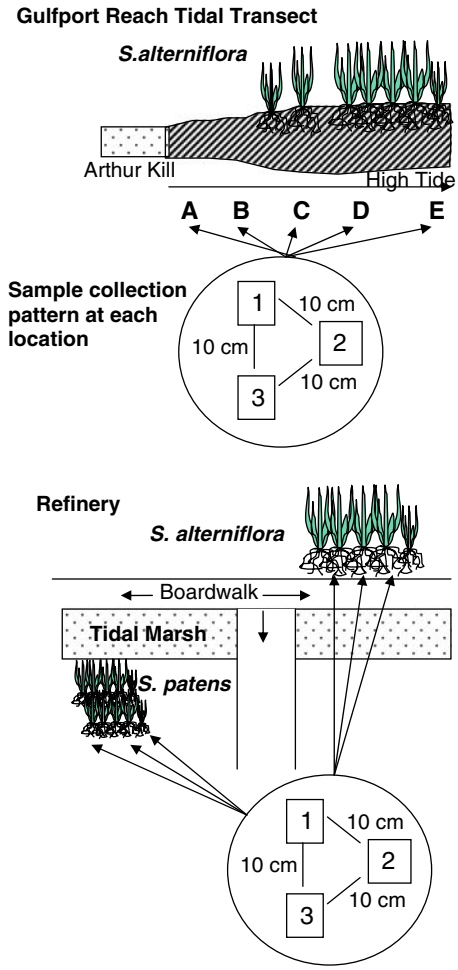

Fig. 1 Conceptual schematic of sampling locations and the pattern of sample collection for ^{14}C-pyrene and ^{14}C-phenanthrene degradation assays and for Most Probable Number assays. The distance from A–E at the Gulfport Reach was approximately 25 m. Three separate samples were collected from each location (A–E) according to the sample collection pattern indicated. The samples collected at the Refinery site were from areas vegetated with *S. alterniflora* or *S. patens*, adjacent to a tidal creek. For the activity measurements three separate samples from one location were sampled, each approximately 10 cm apart. For the MPN analysis three locations, each approximately 1 m apart were sampled, and from each location three separate samples were collected from a distance of approximately 10 cm apart

immediately frozen at −80°C until later analysis. Samples for ^{14}C-PAH degradation and MPN analysis were stored at 4°C overnight and then setup for analysis. Samples for isolation of PAH-degrading bacteria were stored at 4°C for a maximum of five days until isolation studies began.

Media

Minimal salts broth (MSB)

Stanier's minimal salts medium (Stanier et al. 1966) was used with the following modifications: the concentration of $(NH_4)_6Mo_7O_2{\cdot}4H_2O$ was 25 μM, $FeSO_4{\cdot}7H_2O$ was 7 μM, and the $MnSO_4{\cdot}H_2O$ was 9 μM. In addition, nicotinic acid and L-Arginine were added to concentrations of 8 μM and 0.2 mM, respectively). The pH of this medium was approximately 7.0.

Minimal marine broth

This media was based on the Difco (Difco Laboratories 1998) recipe for Marine Media 2216, but was modified in a number of ways such that the final composition per liter of distilled water was: 0.55 g KCl, 0.16 $NaHCO_3$, 0.022 Boric acid, 1.8 g KBr, 0.034 g Strontium Chloride, 0.0024 g NaF, 0.002 g $FeCl_3{\cdot}6H_2O$, 0.0008 g Na_2HPO_4, 0.002 g NH_4NO_3, 19.45 g NaCl, 5.9 g MgCl, 0.068 Na_2SO_4. This medium was prepared at either 5% or 50% of full-strength concentration to a final pH of 7.0±0.5. Tryptic Soy Agar and Marine Media 2216 were purchased from Difco Laboratories.

Chemicals

All PAHs were purchased from either Sigma Aldrich or Fisher Scientific at the highest available purity; typically 95% or greater. Other reagents were laboratory or technical grade (Sigma Aldrich or Fisher Scientific).

Isolation methods

Bacterial strains were isolated using both direct isolation and enrichment techniques, as described in Daane et al. (2001) with the exception of varying the minimal medium type (see Table 1). Phenanthrene or naphthalene were provided as the sole carbon source for both direct isolation and enrichment strategies. Phenanthrene and naphthalene were added directly to liquid growth medium to a final concentration of 500 mg/l. For agar plates phenanthrene was provided as an agar overlayer and naphthalene was provided in the vapor phase (plates incubated in a sealed jar with naphthalene crystals).

Enumeration of total heterotrophic bacteria was conducted in the same manner as the direct isolation experiments except samples were plated both on minimal medium plus PAH as carbon source, and onto Tryptic Soy Agar and Difco Marine Media 2216.

Bacterial identification by FAME analysis

PAH-degrading isolates were identified by analysis of fatty acid methyl ester profiles (FAME) using the Sherlock Microbial Identification System (MIDI, Inc., Newark, DE). Pure cultures of each isolate were grown at 28°C for 24 h on tryptic soy agar plates. Cell samples were then collected, saponified, methylated, extracted and analyzed by gas chromatography as described in the Sherlock Microbial Identification System instructions. Cells that grew slowly were allowed to grow for 48 h, or replicate plates were grown for 24 h. Fatty acid methyl ester profiles obtained by GC analysis were compared with the TSBA (version 3.9) Aerobe database of the MIDI system and matched using unweighted-pair matchings in the dendrogram program of the software provided.

16S rRNA gene sequencing

The FAME-based identification of several isolates was confirmed by partial 16S rRNA gene analysis. DNA was isolated from bacterial strains grown on either TSA or minimal medium plates (with PAH as the carbon source, as per isolation methods). Approximately one culture loop was sampled from a young culture and frozen at −20°C. Samples were then thawed at a later time and incubated with 1 ml of 1.5 mg/ml lysozyme solution (prepared in minimal medium) at 37°C for 1 h with periodic vortexing. Genomic DNA was extracted as described by Sambrook et al. (1989), and the 16S rRNA gene was amplified using the 27F and 1522R primers described in Johnson (1994). The PCR mixtures (50 μl)

Table 1 Isolation, identification and aromatic hydrocarbon utilization of aerobic bacteria from salt marshes of the NY/NJ Harbor

Isolate	Identification[a]		Isolation conditions[b]				PAHs degraded in liquid culture[c]			Aromatic compounds used as growth substrates[d]
	Identification	Similarity (%)	Location	PAH	Medium	Heat	PHE	FLU	FLUOR	
Brevundimonas										
XPG12[e]	*B. vesicularis*	0.114	*S. patens* (Refinery)	PHE di	50%	N	70 ± 9.1[f]	73 ± 7.3[f]	0[f]	NAP, BIP, TOL, M-XY, indole = blue
XPG61	*B. vesicularis*	0.134	*S. patens* (Refinery)	PHE	50%	N	0[f]	88 ± 3[f]	0[f]	NAP, BIP, TOL, M-XY, BENZ, PHE, IND = brown/purple
Flavobacterium										
XPG8	*F. ferrugineum*	0.079	*S. patens* (Refinery)	PHE di	50%	N	46 ± 5[f] 95 ± 0[g]	63 ± 4[f]	0[f]	NAP, BIP, TOL, M-XY, BENZ, PHEN
Paenibacillus										
GPRP1	*P. validus*	0.92	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, PHEN
GPRP4a[e]	*P. validus*	0.934	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, M-XY, Phen
GPRP5b	*P. gordonae*	0.729	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, PIB, BENZ
GPRP06	*P. validus*	0.763	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, BENZ, PHEN
GPRP07[e]	*P. validus*	0.878	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	BIP, PHEN
GPRP11[e]	*P. validus*	0.724	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, PHEN, IND = pink
GPRP12	*P. validus*	0.937	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, PHE
GPRP14	*P. validus*	0.855	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, M-XY, PHE
GPRP16	*P. validus*	0.916	*S. alt* (GPR-C)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, PHE
GPRP29[e]	*P. validus*	0.521	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, M-XY, PHE
GPRP38	*P. validus*	0.886	Bulk Sed (GPR-B)	PHE	MSB	N	100[g] 20 ± 10[f]	0[d]	0[d]	NAP, BIP, PHE
GPRP42f[e]	*P. validus*	0.903	Bulk Sed (GPR-B)	PHE	MSB	N	100[g] 0[f]	27 ± 7[d]	0[d]	NAP, BIP, PHE, IND = pink
GPRP48	*P. validus*	0.911	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, PHE
GPRP49b	*P. validus*	0.908	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, M-XY, PHE
GPRP50	*P. validus*	0.932	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	ND
GPRP52	*P. validus*	0.903	Bulk Sed GPR-B)	PHE	MSB	Y	100[g]	ND	ND	ND

Table 1 continued

Isolate	Identification[a]		Isolation conditions[b]				PAHs degraded in liquid culture[c]			Aromatic compounds used as growth substrates[d]
	Identification	Similarity (%)	Location	PAH	Medium	Heat	PHE	FLU	FLUOR	
GPRP52a	*P. validus*	0.84	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, PHE
GPRP52b	*P. validus*	0.834	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, PHE
GPRP85	*P. validus*	0.644	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	NAP, BIP, TOL, M-XY, PHE
B1EPHSC	*P. validus*	0.788	Bulk Sed (GPR-B)	PHE	MSB	Y	100[g]	ND	ND	ND
GPRN78	*P. validus*	0.233	*S. alt* (GPR-C)	NAP	MSB	Y	16 ± 1[f]	20 ± 10[f]	0[f]	NAP, BIP
GRPN23	*P. thiaminolyticus*	0.404	*S. alt* (GPR-C)	NAP	MSB	Y	ND	ND	ND	NAP, BIP, TOL, M-XY, IND = pink
GPRN25	*P. polymyxa*	0.38	*S. alt* (GPR-C)	NAP	MSB	Y	ND	ND	ND	NAP, IND = pink
GPRN26	*P. validus*	0.231	*S. alt* (GPR-C)	NAP	MSB	Y	ND	ND	ND	NAP, BIP, TOL, M-XY, BENZ, PHE
GPRN75	*P. validus*	0.24	*S. alt* (GPR-C)	NAP	MSB	Y	ND	ND	ND	NAP, BIP, TOL, BENZ
GPRN77	*P. polymyxa*	0.257	*S. alt* (GPR-C)	NAP	MSB	Y	ND	ND	ND	NAP, BIP, TOL, M-XY
GPRN86	*P. validus*	0.664	*S. alt* (GPR-C)	NAP	MSB	N	ND	ND	ND	NAP, BIP, TOL, M-XY, BENZ, PHE, IND = pink
Pseudomonas										
GPRP143	*P. putida*	0.660	*S. alt* (GPR-D)	PHE	MSB	Y	0[f]	0[f]	23.7 ± 4.6[f]	M-XY, PHE
GPRN7	*P. putida*	0.616	*S. alt* (GPR-C)	NAP	MSB	N	13 ± 0[f]	19 ± 2[f]	0[f]	NAP
GPRN8	*P. chloraphis*	0.527	*S. alt* (GPR-C)	NAP	MSB	N	31 ± 4[f]	52 ± 4[f]	0[f]	NAP, IND = blue
GPRN12a	*P. pseudoalcaligenes*	0.826	*S. alt* (GPR-C)	NAP	MSB	N	16 ± 2[f]	28 ± 7[f]	0[f]	NAP, IND = blue
GPRN13	*P. mendocina*	0.791	*S. alt* (GPR-C)	NAP	MSB	N	19 ± 1[f]	29 ± 9[f]	0[f]	NAP, IND = blue
GPRN14	*P. pseudoalcaligenes*	0.870	*S. alt* (GPR-C)	NAP	MSB	N	0[f]	20 ± 13[f]	0[f]	NAP, M-XY, IND = blue
GPRN15b	*P. pseudoalcaligenes*	0.765	*S. alt* (GPR-C)	NAP	MSB	N	0[f]	38 ± 2[f]	0[f]	NAP, IND = blue
GPRN16b	*P. stutzeri*	0.819	*S. alt* (GPR-C)	NAP	MSB	N	0[f]	24 ± 12[f]	0[f]	NAP, IND = blue
GPRN24b	*P. pseudoalcaligenes*	0.794	*S. alt* (GPR-C)	NAP	MSB	Y	31 ± 7[f]	48 ± 7[f]	0[f]	NAP
GPRN24c	*P. pseudoalcaligenes*	0.842	*S. alt* (GPR-C)	NAP	MSB	Y	29 ± 7[f]	41 ± 4[f]	0[f]	NAP, IND = blue
GPRN65	*P. chloraphis*	0.564	*S. alt* (GPR-C)	NAP	MSB	N	17 ± 0[f]	16 ± 1[f]	0[f]	NAP, IND = blue
GPRN66	*P. putida*	0.553	*S. alt* (GPR-C)	NAP	MSB	N	0[f]	18 ± 3[f]	0[f]	NAP, IND = blue
GPRN67	*P. chloraphis*	0.714	*S. alt* (GPR-C)	NAP	MSB	N	26 ± 4[f]	38 ± 5[f]	0[f]	NAP, IND = blue
GPRN68	*P. chloraphis*	0.560	*S. alt* (GPR-C)	NAP	MSB	N	14 ± 4[f]	19 ± 3[f]	0[f]	NAP, BIP, TOL, IND = blue/brown
GPRN84b	*P. pseudoalcaligenes*	0.831	*S. alt* (GPR-C)	NAP	MSB	N	0[f]	39 ± 17[f]	0[f]	NAP

Table 1 continued

Isolate	Identification[a]		Isolation conditions[b]				PAHs degraded in liquid culture[c]			Aromatic compounds used as growth substrates[d]
	Identification	Similarity (%)	Location	PAH	Medium	Heat	PHE	FLU	FLUOR	
GPRP78a	*P. balearica*	0.85	Bulk Sed (GPR-B)	PHEN	MSB	Y	60 ± 41[g]	ND	ND	PHE
Sphingomonas										
GPRP22	*S. capsulatus*	0.181	*S. alt* (GPR-C)	PHE	MSB	N	100[g]	ND	ND	NAP, BIP, TOL, PHE, IND = blue
No Match										
XPG10	No Match	NA	*S. patens (Refinery)*	PHE di	50%	N	90 ± 0[g] 0[f]	41 ± 1[f]	0[f]	NAP, BIP, TOL, M-XY, BENZ, PHE

[a] Identification by Sherlock Microbial Identification System (MIDI). Similarity values are assigned by analysis against the TSBA Aerobe Library

[b] Locations were either Refinery or Gulfport Reach (GPR) with position on the tidal transect (GPR) indicated by a letter A–E

[c] PAH used in the isolation of the strain or for degradation assays is abbreviated as follows PHE = phenanthrene, NAP = naphthalene, FLU = fluorene, FLUOR = fluoranthene. "Medium" column refers to medium used in isolation of the strain and is either MSB (Minimal Salts Broth) or 50% (50% strength Minimal Marine Broth), both described in the Materials and methods. Heat treatment (pasteurization) prior to isolation is indicated by a Y = yes, otherwise N = No in the sub-column "Medium". di = direct isolation. Otherwise isolate obtained after initial enrichment on PAH

[d] Aromatic hydrocarbons tested as growth substrates by provision in the vapor phase to cells growing on agarose plates. Hydrocarbons included naphthalene (NAP), biphenyl (BIP), toluene (TOL), m-xylene (M-XY), and benzene (BENZ). Some strains able to grow on naphthalene were tested for the ability to oxidize indole to indigo (Ensley et al. 1983), blue indicates presence of naphthalene dioxygenases, other color formation also indicated

[e] Identification was also conducted by partial 16S rRNA gene sequencing and BLAST search as described in the Materials and methods. GPRP4a, GPRP07, GPRP11, GPRP29 and GPRP42f were all identified as *P. validus* by both FAME and 16S rRNA partial gene analysis. XPG12 was identified as *Sphingomonas subarctica* by BLAST analysis of partial 16S rRNA gene, and as *B. vesicularis* by FAME analysis

[f] Tested in cocktail of PAHs, as described in Materials and methods

[g] Tested as sole PAH in the medium, as described in Materials and methods

[h] There was some indication in liquid culture assays of possible pyrene degradation, however, when re-tested with ^{14}C-pyrene no degradation was observed.

contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM of each deoxynucleoside triphosphate, 50 pmoles of each primer and 2.5 units of Taq DNA polymerase (Gibco-BRL, Grand Island, N.Y.). Amplifications were performed in a Perkin-Elmer GeneAmp PCR System 2400 thermocycler (Applied Biosystems, Foster City, CA). Reaction conditions were: 96°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final 10 min. extension at 72°C. The gene product of the PCR reaction was purified using the QIAquick PCR Purification Kit (Qiagen). Sequencing reactions were performed using the purified 16S rRNA gene product and 27F or 685R primers (Johnson 1994) on approximately 10 ng of DNA. The sequencing reactions were conducted using Amplitaq FS (Applied Biosystems). PCR reaction conditions were: 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, all for 25 cycles, using a Perkin-Elmer GeneAmp PCR System 2500 thermocycler. Reaction products were analyzed using an ABI Prism 377–Perkin Elmer automated DNA sequencer. Partial 16S rRNA gene sequences obtained were identified using an NCBI Blast Search.

Testing of bacterial isolates for ability to degrade PAHs: liquid culture and plate screening

Five ml cultures of isolates were established in minimal medium (see Table 1). *Paenibacillus validus* PRP1 (Daane et al. 2001) was used as a positive control strain, and one selected test isolate was autoclaved prior to addition of PAHs and used as a killed cell control. Fluorene, phenanthrene, flouoranthene and pyrene were added either individually or as a mixture to a final nominal concentration of 50 mg/l each (through addition of 100 μl of a 2500 mg/l stock solution prepared in dimethyl formamide). Cultures were incubated on a rotary shaker at 125 rpm, room temperature, for 7 days. At this time 2,3-dimethyl-naphthalene was added as an internal standard to each culture (to a final concentration of 5 mg/l), cultures were vigorously shaken by hand, and 10 ml of a 2:1 mixture of hexane and acetone was added. Cultures were then extracted by shaking on a wrist-action shaker overnight. To aid in separation of extraction solvent the cultures were then frozen at −20°C for 12–24 h. The solvent was then removed from the top of the frozen culture and analyzed by gas chromatography-mass-spectrometry (GC-MS) with selected ion monitoring (SIM). The m/z ratios specifically monitored for were 166 (fluorene), 178 (phenanthrene), 202 (flouoranthene and pyrene). The GC-MS system was a Hewlett Packard G1800C GCD Series II with an electron ionization detector, equipped with a Hewlett Packard DB-5 column (30 m in length, 0.25 mm internal diameter). The carrier gas was helium maintained at a column flow of 1.0 ml/min. The initial temperature of the column was 70°C, which was increased at a rate of 30°C/min for 2 min, and then 20°C/min to a final column temperature of 280°C. Data were analyzed using HPChem software. PAH concentrations were determined from a five-point calibration curve and normalized to sediment dry weight.

Degradation of ^{14}C-phenanthrene and ^{14}C-pyrene

Sediment samples collected from the Gulfport Reach tidal transect during June of 2000 and from vegetated sediment of both *S. alterniflora* and *S. patens* growing at the Refinery Site were tested for the ability to mineralize 9-^{14}C-phenanthrene (Sigma-Aldrich, specific activity 11.3 mCi/mmol, radiochemical purity $\geq$95%) and [4,5,9,10]-^{14}C-pyrene (Sigma-Aldrich, specific activity 55 mCi/mmol, radiochemical purity $\geq$95%). Sediment samples (0.7 g of sediment plus 7.3 ml of minimal medium (MSB)) were incubated with either 0.0045 μCi of ^{14}C-phenanthrene or 0.0091 μCi ^{14}C-pyrene plus 5 mg/l of the same non-radiolabeled PAH. Samples were incubated in 20 ml disposable glass test tubes fitted with a suspended 2 ml glass vial containing 1.5 ml of 0.5 M NaOH solution to trap CO_2. The 0.5 M NaOH solution was sampled directly through an 18½ G needle connected to a piece of silicone tubing within the vial. Samples were added to 15 ml of Fisher BD biodegradable liquid scintillation cocktail and analyzed directly on a Beckman LS6500 liquid scintillation counter with automatic quench correction.

Most Probable Numbers determination of phenanthrene-degrading sediment bacterial populations

The number of phenanthrene-degrading organisms in sediment samples from the Gulfport Reach tidal transect and the Refinery Site were quantified using a three-tube MPN test, with degradation of ^{14}C-phen-

anthrene to $^{14}CO_2$ as the endpoint. Serial dilutions of three separate samples collected from each sampling location (Fig. 1) were prepared in minimal salts broth, each receiving 0.0045 μCi of ^{14}C-phenanthrene and 5 mg/l of non-radiolabeled phenanthrene. Samples were incubated with the $^{14}CO_2$-trapping systems described above, and analyzed after one and four weeks of incubation. Vials were gently hand shaken and vortexed daily to prevent conditions from becoming anaerobic. Vials with greater than 500 dpm (2.25×10^{-4} μCi which constitutes 5% of the added radioactivity to each sample) in the CO_2 trap were scored positive for ^{14}C-phenanthrene degradation. This cutoff value for positive scores was chosen based on the radiochemical purity of phenanthrene ($\geq$95%). Any amount of radioactivity greater than 5% of the added radioactivity can be assumed to be due to mineralization of the phenanthrene itself and not due to mineralization of trace impurities in the substrate. The most probable number of degraders was then estimated using the MPN tables of DeMan (1975).

Microbial community analysis using PLFA profiling

Frozen sediment samples (10 g) collected from the Gulfport Reach during May, June and November of 2000 were thawed in a room temperature water bath for 2 h before extraction with 20 ml of a single phase solvent consisting of chloroform: methanol: 0.05 M phosphate buffer (pH 7.4) in a ratio of 1:2:0.8. Samples were extracted overnight on a side-side shaker, and then centrifuged at 1500 g for 15 min. The supernatant and any pockets of chloroform trapped in the sample were collected, and the sample was extracted again with 10 ml of extraction solvent for an additional 2–3 h. Chloroform and phosphate buffer (2.4 ml each) were then added to the combined extraction solvent, and the samples were centrifuged for 10 min at 1500 g and incubated at 4°C overnight. The chloroform fraction was purified using silicic acid (100–200 mesh) columns (0.5 g in a Pasteur pipette). The columns were loaded with the chloroform fraction, and then washed with 5 ml of chloroform, followed by 10 ml of acetone. The phospholipid fatty acids were then eluted from the column in 5 ml of methanol which was evaporated using a LabConco Evaporation system (1.5 h, 55°C, 200 rpm). The dried fatty acids were saponified, methylated, extracted and analyzed by gas chromatography using the procedures of the Sherlock Microbial Identification System (MIDI, Inc., Newark, Del.). The resulting chromatographic data were analyzed by principal component analysis using the Minitab Release II program (State College, PA, USA).

16S rRNA gene TRFLP analysis

DNA was extracted from sediments and purified using the methods of Scala and Kerkhof (1998). PCR with 10 ng of genomic DNA was carried out by using the 27 F (fluorescently labeled) and 1525 R primers, and standard conditions as described by Sakano and Kerkhof (1998). PCR products were gel quantified and then subjected to restriction digestion for 2 h at 37°C with MnlI (New England Biolabs, Inc., Beverly, Mass.). Samples were run on an ABI 373A automated sequencer. TRFLP were determined by comparing with internal standards using the GeneScan software (Perkin-Elmer).

Results

Isolation, identification and characterization of PAH-degrading bacterial isolates

Using PAH as a sole carbon source and various isolation and enrichment methods 48 bacterial isolates were obtained from vegetated and non-vegetated sediments of *Spartina*-dominated salt marshes in the NY/NJ Harbor using naphthalene or phenanthrene as a sole carbon source. The isolates are presented in Table 1 and included isolates from the genera *Paenibacillus*, *Pseudomonas*, *Brevundimonas* and *Flavobacterium*. Isolation conditions and patterns of hydrocarbon-degradation are also provided in Table 1.

The majority of the PAH-degrading isolates were from the genera *Paenibacillus* and *Pseudomonas* (Table 1). A greater number and diversity of isolates were obtained from the vegetated sediments than the non-vegetated sediments (Table 1), with 37/48 (77%) of hydrocarbon-degrading isolates were from the rhizosphere of either *S. patens* or *S. alterniflora.* Only 11/48 isolates were obtained from the non-vegetated sediment and most of these were identified as *Paenibacillus validus*. The majority of the *Paeniba-*

cillus strains were isolated from heat-treated sediment samples on phenanthrene as a sole carbon source. The majority of all isolates were obtained after initial enrichment rather than direct isolation, suggesting that the proportion of PAH-degrading organisms, relative to the total microbial community in these salt marsh sediments is low.

The FAME profiles of selected *Paenibacillus* spp. were analyzed using a pairwise-comparison Euclidean-distance dendrogram presented in Fig. 2. Several reference strains including *Paenibacillus validus* (ATCC BAA-206^T), *P. naphthalenovorans* (PR-N1, ATCC 43897^T), *P. alvei* (ATCC 6344^T), *P. polymyxa* ATCC 842^T) and *P. pabuli* ATCC 43899^T) were used to determine the sub-grouping of the *Paenibacillus* strains. Overall, *Paenibacillus* isolates grouped either with *Paenibacillus validus* or with *Paenibacillus naphthalenovorans* as observed by Daane et al. (2001). Typically, the *Paenibacillus* isolated on naphthalene as the sole carbon source (identified as

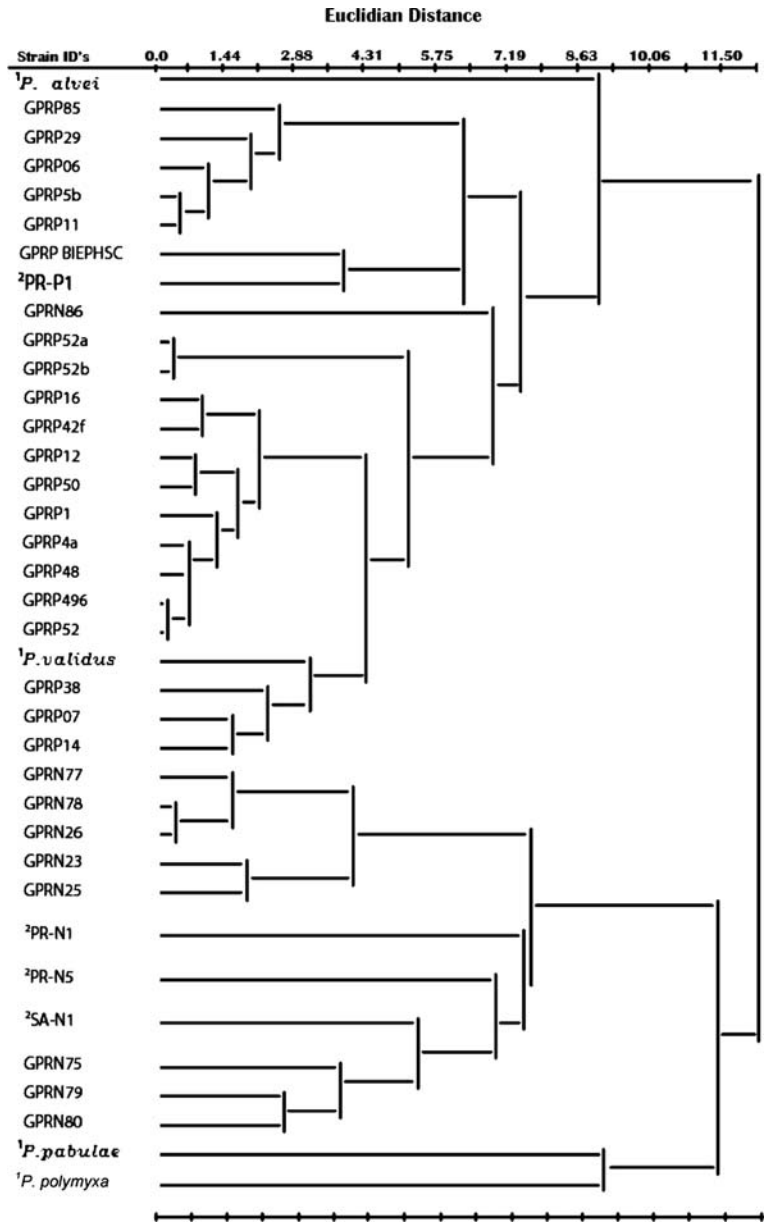

Fig. 2 Dendrogram of selected *Paenibacillus* spp. isolated from the Gulfport Reach sampling site based on fatty acid methyl ester profile analysis (see methods). Superscript one denotes reference strains. Superscript two *Paenibacillus* spp. isolated by Daane et al. (2002)

"GPRNXX") grouped with *Paenibacillus napthalenovorans*. *Paenibacillus* strains isolated on phenanthrene as the sole carbon source grouped with *Paenibacillus validus*.

Several *Paenibacillus* stains were also subjected to partial 16S rRNA gene sequencing and identification by BLAST search. The strains sequenced were: GPRP4a, GPRP07, GPRP11 and GPRP42f. All were identified as *P. validus* by BLAST search, validating the FAME-based identification.

Mineralization of ^{14}C-phenanthrene and ^{14}C-pyrene degradation in sediments

Gulfport Reach site

All samples collected from the Gulfport Reach contained aerobic microbial communities able to mineralize ^{14}C-phenanthrene to $^{14}CO_2$, with mineralization levels ranging from 16–35% (Fig. 3A, Table 2). Mineralization began within one day of incubation in all samples (Fig. 3A). The greatest level of mineralization occurred in samples collected from the most vegetated location (point E) where 35.4 ± 3.3% of the initial radioactivity added as ^{14}C-phenanthrene was mineralized to $^{14}CO_2$ within 35 days. In contrast, only 16–18% of the ^{14}C-label was mineralized by 38 days in samples collected from either points A or B, the non-vegetated sediment locations (Fig. 3A, Table 2). At the conclusion of the experiment (t = 35 days) sample locations could be ranked from least to greatest level of ^{14}C-phenanthrene mineralization as B < A < C < D < E (Fig. 3A, Table 2).

Microbial communities in all sediment samples collected from the Gulfport Reach site were able to mineralize ^{14}C-pyrene with mineralization levels ranging from 2.9 ± 0.6–9.7 ± 1.1% within 35 days of incubation (Fig. 3B, Table 2). The greatest level of ^{14}C-pyrene mineralization occurred in the most sparsely vegetated sampling location (point C), and the least mineralization occurred in the non-vegetated sample collected from point B. Sample locations could be ranked from least to greatest level of ^{14}C-pyrene mineralization as A < B < D < E < C. Pyrene mineralization did not begin immediately in any of the samples. Instead, a lag time ranging from approximately two days (point C) to 4 days (points D and E) was observed (Fig. 3B).

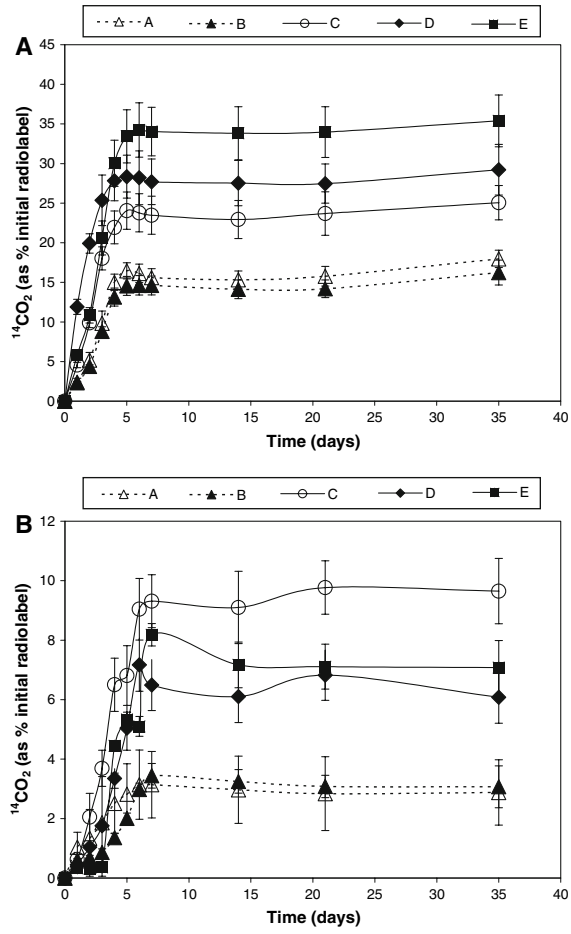

Fig. 3 Ability of microbial communities present in samples from a tidal transect in the Gulfport Reach site to mineralize ^{14}C-phenanthrene (**A**) and ^{14}C-pyrene (**B**) in aerobic cultures. The average and standard error of $^{14}CO_2$ evolution from three replicate samples are presented. Data are the percent of initial ^{14}C added as PAH. Sampling locations are described in the Materials and methods and statistical comparison of day 35 averages are presented in Table 2

The data is limited by the number of replicate samples collected at each point (3), and by the close proximity of replicate samples (samples were collected within 10 cm of one another at each sampling location, see Fig. 1A), however the pattern of results is generally supported by pairwise t-tests (see Table 2) and further supported by the results of the MPN analysis (see below).

Refinery site

Evaluation of the ^{14}C-phenanthrene and ^{14}C-pyrene degradation potential of vegetated sediments col-

Table 2 Comparison of ^{14}C-phenenathrene and ^{14}C-pyrene mineralization by 35 days of culture in samples from the Gulfport Reach tidal transect by pairwise *t*-test

Sample & PAH[a]	Average ± SE[b]	Comparison[c]	*t* calculated[d]	*p* calculated[d]
Gulfport Reach—Phenanthrene				
A	17.96 ± 1.10	A vs. B	0.90	0.43
B	16.23 ± 1.58	A vs. C	2.41	0.10
C	25.07 ± 2.74	A vs. D	3.33	0.04
D	29.22 ± 3.20	A vs. E	5.05	0.01
E	35.39 ± 3.27	B vs. C	2.79	0.09
		B vs. D	3.97	0.03
		B vs. E	5.27	0.07
		C vs. D	0.98	0.01
		C vs. E	2.42	0.40
		D vs. E	1.44	0.24
Gulfport Reach—Pyrene				
A	2.88 ± 0.59	A vs. B	0.21	0.85
B	3.07 ± 0.70	A vs. C	5.43	0.01
C	9.65 ± 1.10	A vs. D	3.03	0.06
D	6.08 ± 0.87	A vs. E	3.87	0.03
E	7.08 ± 0.91	B vs. C	5.05	0.17
		B vs. D	3.32	0.05
		B vs. E	3.49	0.01
		C vs. D	2.55	0.04
		C vs. E	1.81	0.08
		D vs. E	0.70	0.53

[a] PAH were either phenanthrene or pyrene. Sample locations are described in the Materials and methods and presented in Fig. 1

[b] Average and standard errors of three replicate samples are presented. The data represent the total $^{14}CO_2$ evolved from aerobic cultures after 35 days of incubation (see Fig. 3)

[c] Comparison of means for each sample location indicated

[d] Student *t* values were calculated as described in Sokal and Rohlf (1995). T crit (*t* critical) values were also derived from the table of Student *t* values presented in the same source

lected from sediments vegetated with either *S. alterniflora* or *S. patens* at the Refinery Site was also conducted. Samples from both locations showed the ability to mineralize phenanthrene within two days of incubation under aerobic conditions (Fig. 4A). ^{14}C-phenanthrene mineralization proceeded to a level of 28.8 ± 2.6% within 5 days in sediments vegetated with *S. patens*, in comparision to a level of 19.7 ± 2.2% in sediments vegetated with *S. alterniflora*. A pairwise *t*-test (data not shown) indicated that this difference is not significant at $p = 0.05$.

Samples of sediment vegetated with both plant types also mineralized pyrene to a limited extent (Fig. 4B). In contrast to the results for ^{14}C-phenanthrene mineralization, *S. alterniflora* samples appeared to demonstrate a greater extent of pyrene mineralization than did *S. patens* samples. *S. alterniflora* samples mineralized 6.4 ± 2.1% of the initial ^{14}C-pyrene within 5 days of incubation while *S. patens* samples mineralized only 1.9 ± 0.6%. This difference was not statistically significant at a $p = 0.05$, as determined by pairwise comparison using a student *t*-test (data not shown).

Enumeration of ^{14}C-phenanthrene-degrading microorganisms in sediment microbial communities

Phenanthrene-degrading bacteria were enumerated using a ^{14}C-phenanthrene three tube MPN method in

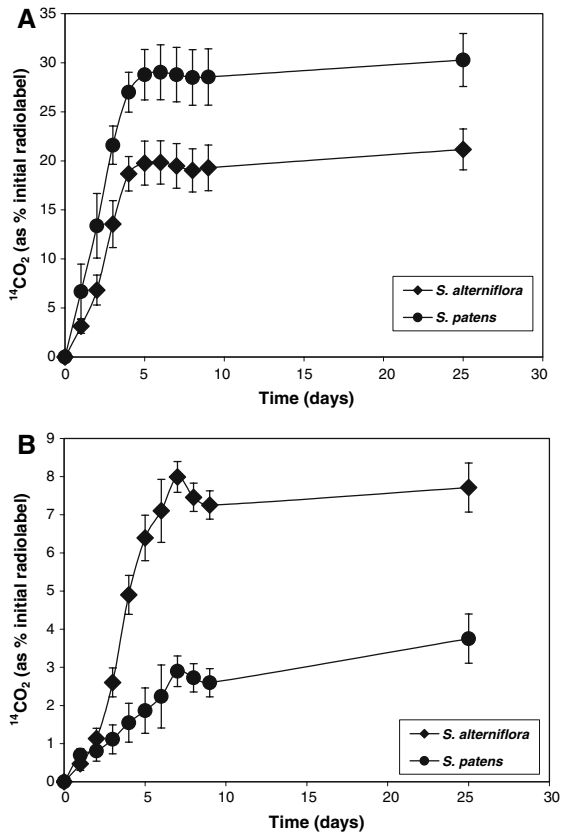

Fig. 4 Ability of microbial communities present in samples vegetated with either *S. alterniflora* or *S. patens* from the Refinery site to mineralize ^{14}C-phenanthrene (**A**) and ^{14}C-pyrene (**B**) in aerobic cultures. The average and standard error of $^{14}CO_2$ evolution from three replicate samples are presented. Data are the percent of initial ^{14}C added as PAH. Sampling locations are described in the Materials and methods. Comparison of PAH degradation by T = 25 days using a pairwise *t*-test revealed that the difference in degradation was not statistically significant between sample types at a $p = 0.05$

samples collected across the tidal transect of the Gulfport Reach and in the rhizosphere of *S. alterniflora* and *S. patens* sampled from the Refinery site (see Table 3 for the Most Probable Number and 95% confidence intervals). At the Gulfport Reach site phenanthrene-degrading bacteria were observed to increase in number as vegetation increased. In the non-vegetated samples from locations A and B, the phenanthrene-degrading bacterial population was estimated to occur at levels of 4×10^2–9×10^2 cells/g sediment wet weight respectively. In contrast in the vegetated samples C, D and E the estimated number of phenanthrene degrading bacteria increased an order of magnitude with each point "up" the tidal transect, from 9×10^3, 4×10^4 and 1.5×10^5 cells/g sediment wet weight, respectively (Table 3).

Only vegetated samples were analyzed at the Refinery site. There, the level of phenanthrene-degrading bacteria in sediments vegetated with *S. alterniflora* ranged from 2.1×10^2 to 9.0×10^4 cells/g sediment wet weight. In sediments vegetated with *S. patens* the population size ranged from 9.0×10^2 to 2.3×10^4 cells/g sediment wet weight. The number of total heterotrophic aerobic bacteria in sediments from both sampling sites was 10^8 cells/g (data not shown, determined using plate counts on both TSA and Difco 2216 Marine Medium). Thus the PAH-degrading bacterial community represented a maximum of approximately 0.01% of the total culturable bacterial community at the vegetated locations sampled, and likely a much lesser proportion of the total culturable bacterial community in the non-vegetated sediment.

Community analysis using PLFA and TRFLP

Unlike the specific PAH-degrading communities, the total microbial communities compared using PFLA analyses, did not show a significant difference when vegetated and non-vegetated sediment profiles were compared (see Fig. 5). The PLFA profiles representing the total microbial communities were similar across the tidal transect samples collected in May and November. However, samples collected in June, when plant growth was peaking in the year 2000, did show some difference between samples collected from point A (closest to the water) versus points B through E. This may have been a result of the high productivity of the vegetation at this time, but other factors, such as sediment temperature or redox in relation to tidal inundation cannot be ruled out as alternative explanations.

Analysis of the terminal restriction fragment length polymorphism patterns between vegetated and non-vegetated samples from the Gulfport Reach samples was conducted for samples from each point on the tidal transect, A through E, collected in May and June of 2000. There was essentially no difference between the non-vegetated samples collected in May and those in June, and likewise no difference in the vegetated samples collected in May and in June (data not shown). A higher number of individual TRFLP

Table 3 Most Probable Number (MPN) estimates for phenanthrene-degrading microorganisms in salt marsh sediments from the NY/NJ Harbor

Sample	MPN[a]	95% Confidence interval
Gulfport reach tidal transect[b]		
A (bulk sediment, nearest water)	4.0×10^2	2.0×10^2–2.1×10^3
B (bulk sediment)	9.0×10^2	3.0×10^2–3.9×10^3
C (rhizosphere sediment, *S. alterniflora*)	9.0×10^3	3.0×10^3–3.9×10^4
D (rhizosphere sediment, *S. alterniflora*)	4.0×10^4	2.0×10^4–2.1×10^5
E (rhizosphere sediment, *S. alterniflora*)	1.5×10^5	5.0×10^4–5.1×10^5
Refinery[c]		
S. alterniflora rhizosphere, A	2.1×10^2	8.0×10^1–6.3×10^2
S. alterniflora rhizosphere, B	4.0×10^4	2.0×10^4–2.1×10^5
S. alterniflora rhizosphere, C	9.0×10^3	3.0×10^3–3.0×10^4
S. patens rhizosphere, A	9.0×10^3	3.0×10^3–3.0×10^4
S. patens rhizosphere, B	9.0×10^2	3.0×10^2–3.0×10^3
S. patens rhizosphere, C	2.3×10^4	7.0×10^3–1.3×10^5

[a] MPN = Most Probable Number. Values are based on the 3-tube MPN tables of DeMan (1975) and represent number of bacteria per gram (wet weight) of sediment sample

[b] See Fig. 1. From the Gulfport Reach location three replicate samples were collected from each location and used for the MPN analysis

[c] See Fig. 1. Refinery samples A, B and C represent three different rhizosphere samples collected approximately 1 m apart within an area of vegetation. Three replicate samples were collected from each of these locations and used to conduct the MPN analysis

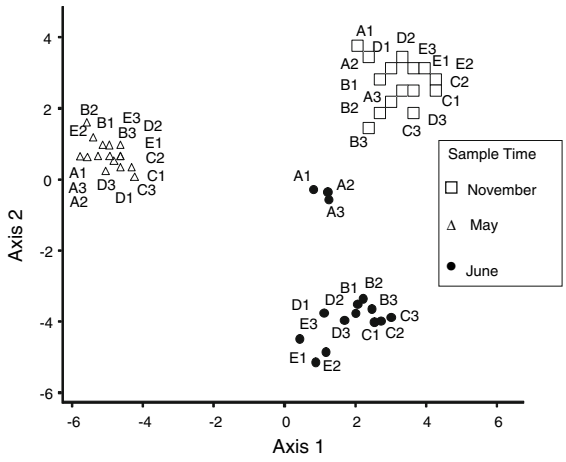

Fig. 5 Principal component analysis of fatty acid methyl ester profiles from microbial communities within locations sampled at the Gulfport Reach of Staten Island during the months of May, June and November of 2000. Locations are described in the Materials and methods

peaks occurred in the non-vegetated sediment profiles suggesting that there may be greater diversity in the microbial community in the non-vegetated sediment than the vegetated sediments. No peaks were evident at either position 87 or 93 base pairs, where peaks correlating to either *Paenibacillus validus* or *Paenibacillus naphthalenovorans* (respectively) were expected based on 16S rRNA gene sequence analysis for Mnl-I cut sites. Given that the PAH-degrading microorganisms likely are only on the order of magnitude of 0.01% of the total community based on MPN analysis, it is not surprising that the PAH-degrading community members would not be visualized with the TRFLP methodology.

Discussion

The data presented herein demonstrate that the sediments vegetated with *Spartina* species occurring in the New York/New Jersey Harbor salt marshes contain aerobic PAH-degrading bacterial communities, including those able to degrade the high molecular weight PAH (i.e., greater than three rings) pyrene.

Forty eight PAH-degrading aerobic bacterial isolates were obtained, the majority from vegetated

sediment at the GPR site which was emphasized in the isolation work undertaken. The majority of PAH-degrading isolates were from the genus *Pseudomonas* and *Paenibacillus*. The isolation of numerous *Pseudomonas* spp. was expected, since PAH-degrading pseudomonads are ubiquitous in soils and sediments, and are well-known petroleum hydrocarbon-degrading bacteria (for example Cerniglia 1992; Zylstra et al. 1997; Berardesco et al. 1998; Daane et al. 2001).

The isolation of numerous *Paenibacillus* spp. from both vegetated and non-vegetated sediments during the present study supports and extends the findings of Daane et al. (2002) who identified the Gram-positive spore-forming *Paenibacillus* spp. as important members of the aromatic hydrocarbon-degrading microbial community in the NY/NJ salt marsh vegetated sediments. The *Paenibacillus* strains isolated in this study grouped taxonomically with either *P. validus* (the phenanthrene-degrading isolates) or with *P. naphthalenovorans* (the naphthalene-degrading isolates), similar to the observations of Daane et al. (2001). The isolation of several *Paenibacillus* strains from the non-vegetated sediments demonstrates that although enriched in vegetated sediments, they are not limited to co-location with plants.

Members of the genus *Paenibacillus* are ubiquitous, existing in many environments including soil, sediment (Siefert et al. 2000) and rhizosphere (Elo et al. 2001; Bent et al. 2002). Several of the Paenibacilli identified in recent studies have the ability to degrade common environmental contaminants, including petroleum hydrocarbons (Pichinoty et al. 1986; Meyer et al. 1999; Daane et al. 2001 and this study), textile and olive mill waste water components (Aguilera et al. 2001), and chlorinated compounds such as biphenyl and dibenzofurans (Pichinoty et al. 1986; Daane et al. 2001; Sakai et al. 2005; Iida et al. 2006; and this study). These findings support the importance of Paenibacilli as members of the aerobic PAH-degrading bacterial community present in both vegetated and non-vegetated sediments in *Spartina*-dominated salt marshes of the NY/NJ estuary.

The MPN analysis results indicated that the aerobic PAH-degrading bacterial communities are one to three orders of magnitude more abundant in vegetated sediments relative to non-vegetated sediments (10^5 cells/g vs 10^2 cells/g respectively, see Table 3 for MPN estimate and relevant 95% confidence intervals). ^{14}C-PAH radiotracer studies also supported a trend towards greater PAH degradation potential in vegetated sediments versus non-vegetated sediments.

The sediment microbial communities present in the Gulfport Reach samples and the Refinery Site samples demonstrated the ability to degrade pyrene when tested in ^{14}C-pyrene microcosm studies. No single pyrene-degrading isolate, however, was identified in this study. High molecular weight PAHs are well-known for their recalcitrance (Keck et al. 1989; Cerniglia et al. 1992). There are relatively few bacterial species, with the exception of members of the genus *Mycobacterium* (for example Heitkamp et al. 1988; Kelley and Cerniglia 1995; Boldrin et al.1993; Wang et al. 1995; Lloyd-Jones and Hunter 1997; Cigolini 2000), able to mineralize pyrene in pure culture. Instead, numerous studies in soil and sediment have demonstrated that degradation of high MW PAHs requires the enzymatic capabilities of a diverse microbial community (Boonchan et al. 2000).

The design of this study included limited replication at the sampling sites utilized and thus we cannot definitively conclude that PAH degradation proceeds to a greater degree in vegetated versus non-vegetated sediments. However, the greater number of PAH-degrading bacterial isolates obtained from vegetated versus non-vegetated sediments, the increased mineralization of PAH in vegetated versus non-vegetated sediments, and the greater number of PAH-degrading aerobic bacteria as estimated using a MPN technique in vegetated relative to non-vegetated sediments all support the role of *Spartina* species in promoting microbial degradation of polyaromatic hydrocarbons present in salt marsh sediments. This data supports the potential for *Spartina*-dominated salt marshes to act as contaminant buffers for estuarine ecosystems such as the NY/NJ Harbor estuary (Jackson and Pardue 1999; Daane et al. 2001, 2002) and the use of natural attenuation as a remedy for the aromatic component of petroleum hydrocarbon contamination entering the marshes.

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