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Plasmid-Mediated Mineralization of Naphthalene, Phenanthrene, and Anthracene

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The well-characterized plasmid-encoded naphthalene degradation pathway in *Pseudomonas putida* PpG7(NAH7) was used to investigate the role of the NAH plasmid-encoded pathway in mineralizing phenanthrene and anthracene. Three *Pseudomonas* strains, designated 5R, DFC49, and DFC50, were recovered from a polynuclear aromatic hydrocarbon-degrading inoculum developed from a manufactured gas plant soil slurry reactor. Plasmids pKA1, pKA2, and pKA3, approximately 100 kb in size, were isolated from these strains and characterized. These plasmids have homologous regions of upper and lower NAH7 plasmid catabolic genes. By conjugation experiments, these plasmids, including NAH7, have been shown to encode the genotype for mineralization of [9-^{14}C]phenanthrene and [U-^{14}C]anthracene, as well as [1-^{14}C]naphthalene. One strain, *Pseudomonas fluorescens* 5RL, which has the complete lower pathway inactivated by transposon insertion in *nahG*, accumulated a metabolite from phenanthrene and anthracene degradation. This is the first direct evidence to indicate that the NAH plasmid-encoded catabolic genes are involved in degradation of polynuclear aromatic hydrocarbons other than naphthalene.

The biochemistry and genetics of the naphthalene degradation pathway contained on plasmid NAH7 have been well characterized and thoroughly reviewed by Yen and Serdar (29). However, not much is known about the substrate specificity of the enzymes of the *nah* operons and whether the *nah*-encoded enzymes are capable of metabolizing higher polyaromatic hydrocarbons (PAHs). Previous reports have stated that the naphthalene degradation pathway is specific for naphthalene and is not involved in degradation of higher PAHs, such as phenanthrene and anthracene (3, 18). Preliminary reports have identified *nahA*-containing pure-culture isolates which degrade naphthalene, phenanthrene, and anthracene (1, 28).

There have been many pure-culture and environmental studies reporting the extensive degradation and mineralization of PAHs, including phenanthrene and anthracene (3–6, 10, 13–16, 19, 24, 26, 27). It has been postulated that 1-hydroxy-2-naphthoic acid is an intermediate of phenanthrene degradation feeding into the naphthalene degradation pathway and oxidized through salicylate and catechol. The 1-hydroxy-2-naphthoic acid may be oxidized through *o*-phthalate and protocatechuate (18).

To our knowledge, there have been no reports of specific enzymes or genes that are responsible for phenanthrene metabolism. We observed that organisms containing NAH7 and NAH7-like plasmids can clear phenanthrene and anthracene spray plates. However, there have been no reports of the *nah* system being able to mediate degradative activity against any PAH other than naphthalene. In this report, we show that NAH7 and NAH7-like plasmids can mediate metabolism of phenanthrene and anthracene as well as naphthalene. In addition, a mutant blocked in the *nahG* (salicylate hydroxylase) gene produced unidentified metabolites when it was grown in the presence of phenanthrene and anthracene. This implies that phenanthrene and anthracene are degraded through the *nah* plasmid-encoded system.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids described in this study are listed in Table 1. All strains were grown on yeast extract-peptone-glucose (YEPG) medium (23) or yeast extract-peptone-succinate-salicylate (YEPSS) medium. YEPSS medium contained (per liter; pH 7.0) 0.2 g of yeast extract, 1.0 g of Polypeptone, 2.7 g of sodium succinate, 0.5 g of sodium salicylate, and 0.2 g of ammonium nitrate. The composition of mineral salts buffer has been described previously (20).

All strains used for mineralization assays were pregrown in 50 ml of YEPG or YEPSS broth overnight at 28°C with shaking (150 rpm). Before use, 25 ml of culture was washed twice in mineral salts buffer by centrifugation at 12,000 × *g* for 10 min at 5°C. Washed cells were resuspended in 1 ml of mineral salts buffer.

Plasmid isolation and analysis. Putative NAH-type plasmids from the environmental isolates were subjected to extensive characterization to identify similarities and differences in the *nah* catabolic genes. Cultures for large-scale plasmid isolation were grown in YEPG broth overnight with shaking (200 rpm) at 28°C. Plasmids were isolated by the procedure outlined in a Promega technical bulletin (22a). Plasmids were further purified by cesium chloride ultracentrifugation followed by butanol extraction and ethanol precipitation (21). Plasmid DNA was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) and stored at −20°C. Plasmid DNA was cleaved by digestion with *Eco*RI or *Pst*I (GIBCO/Bethesda Research Laboratories, Gaithersburg, Md.). Fragments were separated by electrophoresis on a 1% vertical agarose gel at 25 V for approximately 16 h.

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TABLE 1. Bacterial strains and plasmids used in this study

Bacterial strain	Strain derivation	Plasmid	Genotype	Reference
P. putida G7	Wild type	NAH7	*nah*$^+$ *sal*$^+$	8
P. fluorescens 5R	Wild type	pKA1	*nah*$^+$ *sal*$^+$	17
P. fluorescens 5RL	Tn*4331*	pUTK21	*nah*$^+$ *sal lux*$^+$	17
P. fluorescens 18H	Wild type	Cryptic	*nah sal*$^+$	17
P. fluorescens HK44	18H × 5RL	pUTK21	*nah*$^+$ *sal*$^+$ *lux*$^+$	17
Pseudomonas sp. strain DFC50	Wild type	pKA3	*nah*$^+$ *sal*$^+$	This study
Pseudomonas sp. strain DFC49	Wild type	pKA2	*nah*$^+$ *sal*$^+$	This study
P. putida 2440	Wild type		*nah sal*	2
Pseudomonas sp. strain AL3004	2440 × NAH7	NAH7	*nah*$^+$ *sal*$^+$	This study
Pseudomonas sp. strain HK001	2440 × DFC49	pKA2	*nah*$^+$ *sal*$^+$	This study
Pseudomonas sp. strain MRS1	2440 × HK44	pUTK21	*nah*$^+$ *sal lux*$^+$	This study
Pseudomonas sp. strain HK43	2440 × DFC50	pKA3	*nah*$^+$ *sal*$^+$	This study

Agarose gels were blotted onto Biotrans nylon membranes (ICN Biomedical, Costa Mesa, Calif.) by using a VacuGene blotting system (Pharmacia LKB, Piscataway, N.J.) in accordance with the manufacturer's protocol. One buffer (0.5 M NaH_2PO_4, 1 mM EDTA, 7% sodium dodecyl sulfate [SDS]; pH 7.2) was used for prehybridization and hybridization (7).

Probes. The single-stranded *nahA* probe was generated by asymmetric amplification by using *Taq* DNA polymerase (Perkin-Elmer, Norwalk, Conn.) and [α-^{32}P]dCTP (ICN Biomedical). Genes *nahA* through *nahD* of plasmid NAH7 were on a 10-kb fragment in vector pKT230. Radiolabeled probes for *nahA-D* genes ([α-^{32}P]dCTP; ICN Biomedical) were generated by nick translation (GIBCO/Bethesda Research Laboratories). The specific activity of each probe was approximately 10^8 dpm/μg of DNA. A probe with an activity of 10^6 dpm was added to each blot. Membranes were washed under high-stringency conditions (1.17 g of NaCl, 4.84 g of Tris base, 0.74 g of EDTA, and 10 g of SDS in 2 liters [final volume] of distilled water; pH 7.0 to 8.0, adjusted with concentrated HCl) and visualized by autoradiography.

Bacterial conjugation. In order to link the phenanthrene- and anthracene-degradative phenotype to the NAH plasmid, plasmids were transferred to a rifampin mutant *Pseudomonas putida* 2440 recipient (*nah sal*). *Pseudomonas* sp. strains DFC50, DFC49, 5RL, and HK44 and *P. putida* G7 were used as donor strains. Filter matings were performed by using a 1:10 ratio of donor cells to recipient cells. Filters were incubated on Luria-Bertani agar for 24 h at 25°C. Transconjugants were selected on a minimal salts medium containing salicylate (100 mg liter^{-1}) as the sole carbon source and rifampin (50 mg liter^{-1}). In the case of the mating between strains HK44 and 2440, selection was made by plating the preparation on Luria-Bertani agar containing rifampin (50 mg liter^{-1}) and tetracycline (14 mg liter^{-1}). Transconjugants were screened for the appropriate catabolic and antibiotic phenotypes and the *nah* genotype by probing with *nahA*.

Mineralization assay. To test for degradation of PAHs in the wild-type and transconjugant strains, ^{14}C-PAH assays were employed. [1-^{14}C]naphthalene (8.0 mCi/mmol; purity, >98%), [9-^{14}C]phenanthrene (10.4 mCi/mmol; purity, >99%), and [U-^{14}C]anthracene (10.4 mCi/mmol; purity, >98%) were purchased from Sigma Chemical Co., St. Louis, Mo., and were used as supplied. A 2-ml portion of 0.25× YEPSS medium was placed into a 25-ml vial (Pierce Chemical Co., Rockford, Ill.), and an 8-ml vial was used as a CO_2 trap; 0.5 ml of 0.5 N NaOH was placed into the CO_2 trap. Washed cells (final concentration, 10^7 to 10^8 CFU/ml) were added to the mineralization medium. The appropriate ^{14}C-PAH dissolved in acetone (approximately 500,000 dpm) and 50 ppm of unlabeled PAH dissolved in acetone were added, and the vials were sealed with Teflon-lined silicone septa (Pierce). The vials were incubated at room temperature (25°C) with shaking (120 rpm) for 48 h. The negative controls were *P. putida* 2440 (*nah sal*) and *Pseudomonas fluorescens* 18H (*nah sal*$^+$).

The assay was stopped by injecting 0.5 ml of 2 M H_2SO_4 through each septum. After an additional 1 h of shaking, 2 ml of hexane-isopropanol (4:1, vol/vol) was injected through the septum, and the vials were allowed to shake for another 1 h. Then the NaOH and 0.5 ml of the aqueous phase were removed and added to 1 ml of water in separate scintillation vials, and 10 ml of Beckman ReadySafe scintillation fluor was added. The hexane-isopropanol phase (0.2 ml) was added to 10 ml of Econofluor (Dupont) scintillation fluor. The scintillation vials were allowed to sit for 24 h in the dark before they were counted with a Beckman model LS5000 scintillation counter. The H-number method was used for automatic quench compensation and conversion of counts per minute to disintegrations per minute was based on standard quench curves.

Analysis of metabolites from *P. fluorescens* 5RL. The metabolic intermediates produced by *P. fluorescens* 5RL (pUTK21) (*nah*$^+$ *sal*) were compared with the metabolites produced by *P. fluorescens* HK44(pUTK21) (*nah*$^+$ *sal*$^+$). Hexane-isopropanol phases from replicate samples were pooled and evaporated under a gentle stream of nitrogen. The metabolites were reconstituted in 2 ml of acetonitrile (high-pressure liquid chromatography [HPLC] grade), and aqueous phases were filtered through a 0.2-μm-pore-size PTFE (polytetrafluoroethylene) filter (Gelman) prior to HPLC. Samples from both phases were examined for ^{14}C-labeled metabolites by HPLC by injecting 200-μl sample volumes into a Supelcosil LC-18 column (Supelco, Bellefonte, Pa.) (flow rate, 2 ml min^{-1}), with elution for 5 min with water (pH 2.5) followed by a linear gradient to 60% acetonitrile–40% water over a period of 12 min. Detection was accomplished with a photodiode array detector at a wavelength of 255 nm. Eluant from the photodiode array detector was mixed on line with scintillation cocktail (6 ml min^{-1}) and passed through a model IC/CR Flo-One Beta radioactive flow detector (Radiomatic, Tampa, Fla.). The HPLC and the radioactive flow detector were calibrated with [9-^{14}C]phenanthrene and [U-^{14}C]anthracene. The postulated intermediates of phenanthrene and anthracene degradation (1-hydroxy-2-naphthoic acid and 2-hydroxy-3-naphthoic

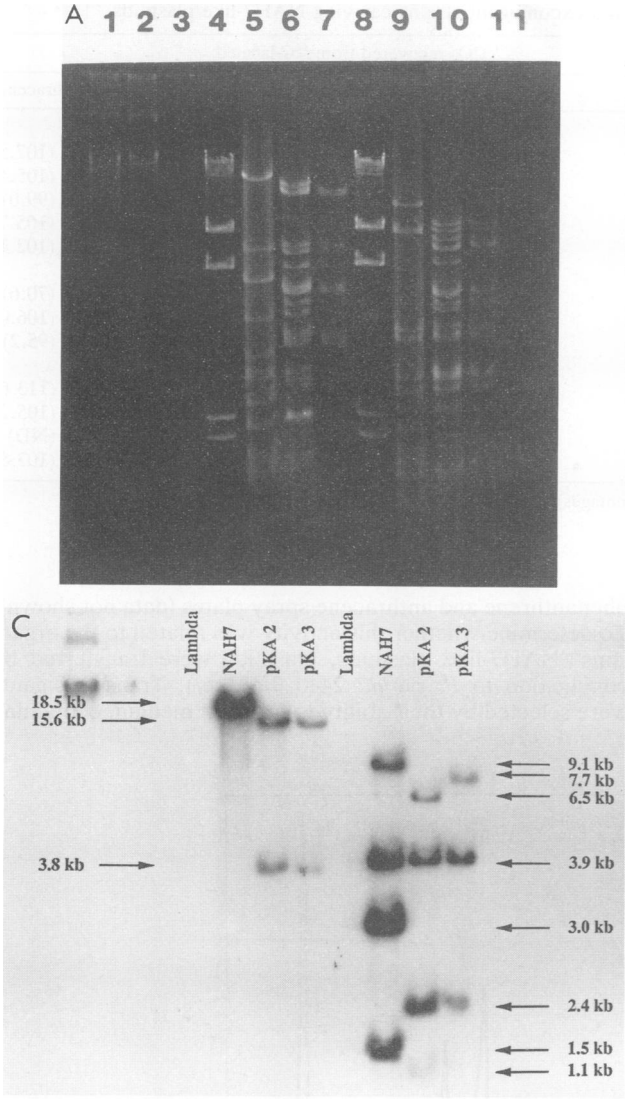

FIG. 1. Molecular relatedness among NAH7 and plasmids recovered from PAH-degrading soil isolates. (A) Restriction digestion of plasmids NAH7, pKA2, and pKA3. Lane 1, NAH7; lane 2, pKA2; lane 3, pKA3; lane 4, *Hin*dIII-digested lambda; lane 5, *Eco*RI-digested NAH7; lane 6, *Eco*RI-digested pKA2; lane 7, *Eco*RI-digested pKA3; lane 8, *Hin*dIII-digested lambda; lane 9, *Pst*I-digested NAH7; lane 10, *Pst*I-digested pKA2; lane 11, *Pst*I-digested pKA3. (B) Southern blot of the gel in panel A probed with ^{32}P-labeled *nahA* DNA fragment. (C) Southern blot of the gel in panel A probed with ^{32}P-labeled *nahA-D* DNA fragment.

acid) were used for comparison with any unknown radiolabeled peaks.

RESULTS

Bacterial strains and plasmids. The wild-type naphthalene-degrading strains listed in Table 1 were isolated from a mixed slurry treatment reactor inoculum for manufactured gas plant soil contaminated with PAHs. These strains were originally isolated by colony hybridization by using pDTG113 (a gift from D. T. Gibson, University of Iowa) as the gene probe and phenanthrene spray plate screening. *P. fluorescens* 5R, DFC49, and DFC50 were found to contain large single-copy plasmids, designated pKA1, pKA2, and pKA3, respectively, in the size range around 100 kb (Fig. 1A). An endonuclease restriction pattern analysis of these plasmids compared with NAH7 revealed significant differences. Plasmid pKA1 was previously reported to have a size and restriction map profile significantly different from those of NAH7 (16); however, the upper and lower catabolic regions of this plasmid are homologous to those of NAH7. Endonuclease restriction digestions of pKA2 and pKA3 with the enzymes *Eco*RI and *Pst*I revealed significant variation in the DNA fragment patterns compared with the pattern generated by NAH7 (Fig. 1A). However, plasmids pKA2 and pKA3 appeared to be the same plasmid, as their *Eco*RI digests generated identical restriction banding patterns (Fig. 1A). *Pst*I digestion of pKA2 and pKA3 revealed a polymorphism between the two plasmids.

Although there were significant differences in the restriction banding patterns of the plasmids on the agarose gel, there were many shared bands. Southern hybridization demonstrated that the endonuclease restriction patterns of the *nah* catabolic genes varied significantly from the restriction pattern of NAH7. Figure 1B shows the results of a Southern hybridization analysis of the gel in Fig. 1A probed with a 1-kb fragment of the naphthalene dioxygenase of NAH7 (*nahA*). The *Eco*RI digestions showed that the *nahA* probe hybridized to an 18.5-kb fragment of NAH7 and a 15.6-kb fragment of pKA2 and pKA3. The *nahA* probe also hybridized to a 3.9-kb fragment of a *Pst*I digestion of all three plasmids, indicating that there was some degree of conservation of the digestion patterns of the catabolic genes.

The same blot was subsequently hybridized with a probe consisting of the *nahA-D* fragment (Fig. 1C). *Eco*RI digestions of pKA2 and pKA3 revealed a new band hybridizing at approximately 3.8 kb, while NAH7 did not produce any new bands hybridizing with the larger probe conforming to a previously published restriction map (29), although there were size anomalies. However, *Pst*I digestions revealed significant differences in hybridization of the *nahA-D* fragment in all three plasmids. In comparison with Fig. 1B, NAH7 had three additional bands hybridizing at 9.1, 3.0, and 1.5 kb (Fig. 1C). Plasmid pKA2 had three additional fragments hybridizing with *nahA-D*, while pKA3 had only two additional fragments. Both pKA2 and pKA3 had a common 2.4-kb fragment, while pKA2 had 6.5- and 1.1-kb fragments

TABLE 2. Levels of mineralization of ^{14}C-PAH by donor and exconjugant strains carrying NAH7-like plasmids

Strain	% $^{14}CO_2$ recovered from ^{14}C-labeled:		
	Naphthalene	Phenanthrene	Anthracene
Donor strains			
P. putida G7(NAH7)	78.3 (96.3)[a]	18.9 (109.3)	13.1 (107.5)
P. fluorescens 5R(pKA1)	77.1 (92.2)	27.6 (99.0)	14.4 (105.4)
P. fluorescens DFC50(pKA3)	78.6 (96.5)	33.7 (105.8)	12.6 (99.0)
P. fluorescens HK44(pUTK21)	68.3 (96.3)	1.6 (125.1)	14.7 (105.7)
P. fluorescens DFC49(pKA2)	63.2 (87.7)	8.4 (97.1)	12.7 (102.1)
Recipient strains			
P. putida 2440	0.0 (104.9)	0.3 (88.6)	0.1 (70.6)
P. fluorescens 18H	0.1 (106.2)	1.2 (131.4)	0.1 (106.0)
Mutant *P. fluorescens* 5RL(pUTK21)	21.6 (104.5)	0.0 (119.5)	13.8 (95.2)
Exconjugant strains			
P. putida HK001(pKA2)	59.2 (121.9)	9.9 (123.9)	56.3 (113.6)
P. putida AL3004(NAH7)	79.1 (97.6)	10.6 (110.9)	14.0 (105.2)
P. putida MRS1(pUTK21)	12.7 (ND)[b]	0.0 (ND)	10.5 (ND)
P. putida HK43(pKA3)	73.3 (99.0)	17.0 (111.1)	11.2 (103.8)

[a] The values in parentheses are values for total ^{14}C recovery (expressed as percentages).
[b] ND, not determined.

and pKA3 had a 7.7-kb fragment. These data indicated that the 6.5- and 1.1-kb fragments are the result of an additional *Pst*I site contained in the 7.7-kb *Pst*I fragment of pKA3.

Previous work had demonstrated that *Pseudomonas* sp. strains 5R, DFC49, and DFC50 and NAH7 were able to clear phenanthrene and anthracene spray plates (data not shown). To determine whether this activity was related to the organisms' NAH7-like plasmids, plasmids were transferred by conjugation to *P. putida* 2440 (*nah sal*). Transconjugants were selected by their ability to grow on medium containing

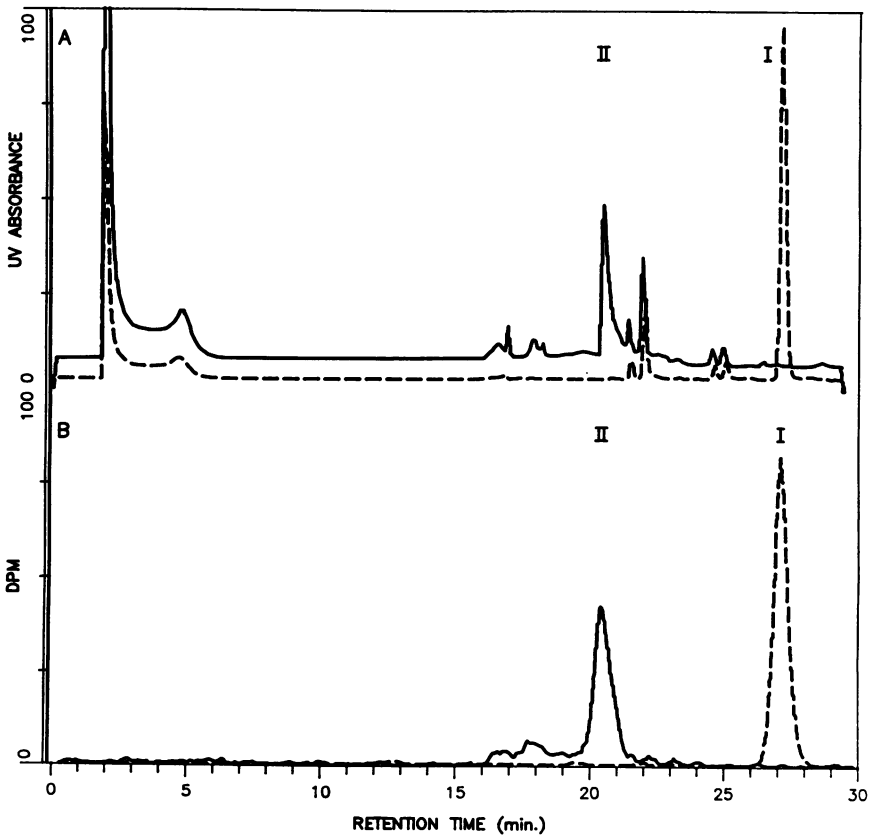

FIG. 2. HPLC chromatogram of metabolites from *P. fluorescens* 5RL grown in the presence of [^{14}C]phenanthrene. [^{14}C]phenanthrene (peak I) produced a radiolabeled metabolite (peak II) with a retention time of 20.4 min. The chromatogram from the control flask (dashed line) is shown along with the chromatogram from the culture supernatant (solid line). (A) UV traces from culture extracts. (B) ^{14}C traces from culture extracts.

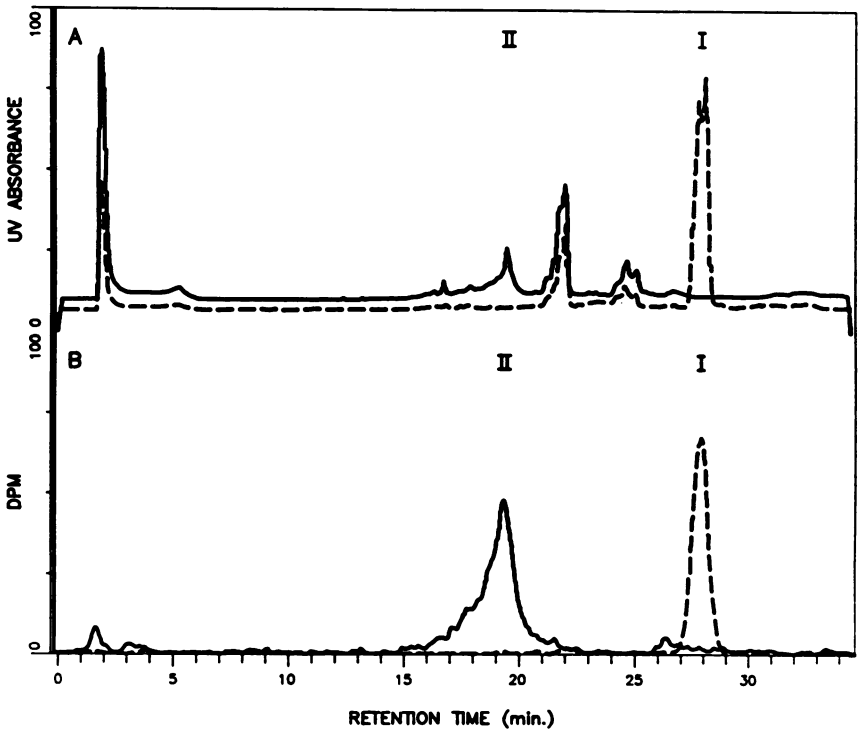

FIG. 3. HPLC chromatogram of metabolites from *P. fluorescens* 5RL grown in the presence of [^{14}C]anthracene. [^{14}C]anthracene (peak I) produced a radiolabeled metabolite (peak II) with a retention time of 19.4 min. The chromatogram from the control flask (dashed line) is shown along with the chromatogram from the culture supernatant (solid line). (A) UV traces from culture extracts. (B) ^{14}C traces from culture extracts.

salicylate, and the *nah* genotype was confirmed by colony hybridization with *nahA* (data not shown). *P. fluorescens* 18H and *P. putida* 2440 did not hybridize with the *nah* gene probes. *P. putida* 2440 does not grow on medium containing salicylate and naphthalene. All resulting transconjugants from the matings with *P. putida* 2440 were tested for their ability to clear phenanthrene and anthracene spray plates. All exconjugants were able to produce zones of clearing with both compounds.

^{14}C-PAH mineralization. A comparison of the abilities of parental and transconjugant strains to mineralize radiolabeled PAHs is shown in Table 2. The data are expressed as percentages of $^{14}CO_2$ recovered from each ^{14}C-PAH added. *P. putida* 2440 and *P. fluorescens* 18H were used as negative controls. All of the wild-type *nah*$^+$ strains produced $^{14}CO_2$ from naphthalene (63.2 to 78.6%), as well as from phenanthrene (8.4 to 33.7%) and anthracene (12.7 to 14.4%). The transconjugants, strains HK001(pKA2), AL3004(NAH7), and HK43(pKA3), were also able to mineralize naphthalene (59.2 to 79.1%), phenanthrene (9.9 to 17.0%), and anthracene (11.2 to 56.3%). The negative control strains produced less than 1.2% $^{14}CO_2$ from any ^{14}C-PAH tested.

Strains HK44 and MRS1 containing plasmid pUTK21 (which is transposon inactivated in the lower pathway) produced $^{14}CO_2$ from [1-^{14}C]naphthalene (68.3 and 12.7%, respectively) and [U-^{14}C]anthracene (14.7 and 10.5%, respectively) because of the position of the radiolabel. The radiolabel in [9-^{14}C]phenanthrene was not accessible, and therefore no $^{14}CO_2$ was detected.

Analysis of metabolites. *P. fluorescens* 5RL(pUTK21), which has an inactivated *nahG*, was grown with naphthalene, phenanthrene, and anthracene in the medium. As reported previously, when this organism was grown on medium containing naphthalene, salicylate accumulated in the medium (17). When this organism was grown with [9-^{14}C]phenanthrene and [U-^{14}C]anthracene, a new radiolabeled metabolite appeared.

When strain 5RL(pUTK21) was grown with [9-^{14}C] phenanthrene, a major radiolabeled peak appeared at 20.4 min (Fig. 2). Analysis of the spectrum of this peak revealed a λ_{max} at 248 nm; this is similar to the spectrum for 1-hydroxy-2-naphthoic acid. When the organism was grown with [U-^{14}C]anthracene, a major radiolabeled peak appeared at 19.4 min (Fig. 3). This was a broad-based peak, which implied that the peak was not a pure compound. A UV scan revealed a nondescript spectrum with a λ_{max} at 270 nm. Conclusive identification of these metabolites has been described by Menn et al. (22).

DISCUSSION

The naphthalene degradation pathway of *P. putida* G7(NAH7) has been well characterized both biochemically and genetically. The phenanthrene metabolic pathway and, to a lesser extent, the anthracene metabolic pathway have been characterized biochemically, but the genes that mediate phenanthrene metabolism have not been identified. Previous reports (3, 18) stated that the naphthalene and phenanthrene pathways are distinct from each other.

Kiyohara and Nagao (18) reported that in 13 different phenanthrene-degrading strains (fluorescent and nonfluorescent pseudomonads, vibrios, and other unidentified strains), the phenanthrene degradation pathway was independent of the lower naphthalene degradation pathway. Growth on

phenanthrene induced an NAH-dependent 2-carboxybenzaldehyde dehydrogenase and protocatechuate oxygenase. Growth on naphthalene induced salicylate hydroxylase and catechol hydroxylase. It was suggested that growth on phenanthrene in all of these strains occurred via protocatechuate and not catechol (18).

Barnsley (3) reported that the initial oxidation of naphthalene and phenanthrene is conducted by two different enzymes even though both compounds can be further metabolized to 1-hydroxy-2-naphthoic acid by a shared set of reactions. He reached this conclusion by comparing the oxidation rates of each substrate in in vitro assays. Ensley and Gibson (9) purified the terminal oxygenase of the naphthalene dioxygenase which binds naphthalene. Unfortunately, phenanthrene and anthracene were not tested as alternate substrates.

The genes that code for naphthalene degradation have been shown to be plasmid encoded and transferrable by conjugation (8). The *nah* genes have been further localized to a 25-kb fragment of plasmid NAH7 by cloning an *Eco*RI fragment of an NAH7::Tn*5* mutant (12, 25). We have shown that these same genes are responsible for the partial degradation of phenanthrene and anthracene, as well as naphthalene.

Plasmid pUTK21 contains a defective *nahG* gene which codes for salicylate hydroxylase. When preinduced cells were grown with phenanthrene and anthracene, a radiolabeled metabolite appeared. This metabolite should be analogous to salicylate, considering the action of the *nahG* gene. The metabolite produced by *P. fluorescens* 5RL from phenanthrene has been tentatively identified as 1-hydroxy-2-naphthoic acid on the basis of its UV spectrum and retention time compared with values for a pure standard. The metabolite produced from anthracene has not been identified but is hypothesized to be 2-hydroxy-3-naphthoic acid, as proposed by Gibson and Subramanian (11). The wild-type and transconjugant strains (except strain HK001), as well as strains containing inactivated *nahG*, produced similar amounts of $^{14}CO_2$ from [U-^{14}C]anthracene.

Although metabolites from the wild-type strains from anthracene mineralization experiments were not analyzed, the metabolite produced from pUTK21-mediated catabolism may be a dead-end product in the wild-type hosts.

The *nah* system or the NAH plasmid may mediate the degradation of more PAHs than previously thought, which implies that maintaining and monitoring one catabolic bacterial population may be sufficient for degradation of a significant fraction of the PAHs in contaminated soil. Kiyohara and Nagao (18) and Barnsley (3) proposed that enzymes other than NahA catalyzed the first step in phenanthrene metabolism. While our study did not prove or disprove this hypothesis, the conjugation and mineralization data suggest that the phenanthrene enzyme is NahA and is on the NAH7-like plasmid. These results do not preclude the existence of alternative biochemical pathways in other bacterial systems.

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