Polycyclic Aromatic Hydrocarbon Degradation by a *Mycobacterium* sp. in Microcosms Containing Sediment and Water from a Pristine Ecosystem

MICHAEL A. HEITKAMP* and CARL E. CERNIGLIA

National Center for Toxicological Research, Food and Drug Administration, Jefferson, Arkansas 72079

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Microcosm studies were conducted to evaluate the survival and performance of a recently discovered polycyclic aromatic hydrocarbon (PAH)-degrading *Mycobacterium* sp. when this organism was added to sediment and water from a pristine ecosystem. Microcosms inoculated with the *Mycobacterium* sp. showed enhanced mineralization, singly and as components in a mixture, of 2-methylnaphthalene, phenanthrene, pyrene, and benzo[a]pyrene. Studies utilizing pyrene as the sole added PAH showed that the *Mycobacterium* sp. survived in microcosms for 6 weeks both with and without preexposure to PAH and mineralized multiple doses of pyrene. Pyrene mineralization rates for sterilized microcosms inoculated with the *Mycobacterium* sp. showed that competition with indigenous microorganisms did not adversely affect survival of or pyrene degradation by the *Mycobacterium* sp. Pyrene mineralization by the *Mycobacterium* sp. was not enhanced by inorganic nutrient enrichment and was hindered by organic nutrient enrichment, which appeared to result from overgrowth of indigenous bacteria. This study demonstrates the versatility of the PAH-degrading *Mycobacterium* sp. and expands its potential applications to include the degradation of two-, three-, four-, and five-ringed PAHs in sediments.

Polycyclic aromatic hydrocarbons (PAHs) occur commonly in the environment as incomplete combustion products of fossil fuels and organic compounds (8, 10). PAHs may be acutely toxic or genotoxic, depending upon the number and configuration of fused benzene rings and the presence or absence of other substituents (for a review, see reference 1). Most higher-molecular-weight PAHs are genotoxic and, due to their low water solubility, are usually bound to suspended particles in aquatic ecosystems and ultimately deposited into sediments (8, 15, 16). Significant levels of PAHs have been reported in sediments from many industrialized areas throughout the world (12).

There is growing interest in the use of microbial technologies to degrade xenobiotic chemicals in the environment (17). However, one major factor determining the success of bioremediation is the availability of microorganisms which possess the catabolic enzymes needed to degrade the chemicals of interest. It is known that selection or adaptation of chemical-degrading microbial populations may occur in the environment as a result of exposure to chemicals (14, 19, 21), but these shifts in microbial populations may occur slowly and are dependent upon the degradability of the chemicals. In the case of PAHs, many genera of bacteria have been reported to utilize lower-molecular-weight PAHs containing two or three fused aromatic rings as the sole sources of carbon and energy (for a review, see reference 1). However, much less is known about the existence of microorganisms and the chemical pathways for the degradation of the more recalcitrant higher-molecular-weight PAHs. Although the mineralization of phenanthrene, a PAH containing three aromatic rings, by a *Mycobacterium* sp. has been reported (2), we recently reported the discovery and characterization of a *Mycobacterium* sp. which was able to extensively degrade PAHs containing up to five fused aromatic rings (5, 6). This *Mycobacterium* sp. was isolated from a eutrophic estuarine ecosystem which has been chronically exposed to petrogenic wastes from an oil field (4). We used the *Mycobacterium* sp. in subsequent studies to investigate the mechanisms for initial enzymatic attack and the chemical pathway for the degradation of pyrene, a PAH containing four condensed aromatic rings (7).

The ultimate usefulness of any chemical-degrading microorganism depends upon its survival and performance in diverse ecosystems. In the present study, we report enhancement of PAH degradation in water and sediments from a freshwater ecosystem after inoculation with the recently discovered PAH-degrading *Mycobacterium* sp. The ecosystem in this report is pristine and normally unable to degrade PAHs containing four or more aromatic rings (4). In addition, we examined the effects of microbial competition, inoculum size, PAH preexposure, and nutrient enrichment on PAH degradation in pristine water and sediments containing the *Mycobacterium* sp.

MATERIALS AND METHODS

Chemicals. Radiolabeled chemicals, specific activities, and sources were as follows: 2-[8-14C]methylphenanthrene, 5.0 mCi/mmole (California Bionuclear Corp., Sun Valley, Calif.); [9-14C]phenanthrene, 19.3 mCi/mmole (Amersham/Searle, Arlington Heights, Ill.); and [4-14C]pyrene, 30 mCi/mmole (Midwest Research Institute, Kansas City, Mo.). Nonlabeled PAH sources were as follows: 2-methylnaphthalene, Chem Service (West Chester, Pa.); phenanthrene and pyrene, Aldrich Chemical Company (Milwaukee, Wis.); benz[a]pyrene, Eastman Organic Chemical Company (Rochester, N.Y.). Chemical analyses by thin-layer chromatography, high-pressure liquid chromatography, and gas chromatography-mass spectrometry showed that the purity of all radiolabeled and nonradiolabeled PAHs exceeded 99%. Glucose and peptone were purchased from Difco.

* Corresponding author.
† Present address: Monsanto Company, St. Louis, MO 63167.
Laboratories (Detroit, Mich.) and GIBCO Diagnostics (Madison, Wis.), respectively. All solvents and inorganic chemicals were of the highest purity available.

**Sediment and water microcosms.** Sediment and water were collected with a Peterson dredge from DeGray Reservoir, near Arkadelphia, Ark. DeGray Reservoir is a pristine manmade impoundment which receives no known significant exposure to chemicals. A detailed description of DeGray Reservoir has been presented previously (3). Biodegradation tests were conducted in a flow-through microcosm test system which permitted continuous monitoring of mineralization and recovery of chemical residues (9). Sediments were homogenized at a low speed in a blender, placed into microcosms, and overlaid with lake water. Microcosms consisted of 0.5-liter glass tanks containing 20 g (wet weight) of sediment and 180 ml of lake water.

**Culture of the PAH-degrading Mycobacterium sp. and inoculation into microcosms.** Pure cultures of the *Mycobacterium* sp. were grown at 24°C in minimal basal salts (MBS) broth containing organic nutrients and pyrene added in *n*,*n*-dimethyl formamide as described previously (5, 6). The optical densities of the cultures at 500 nm (OD500s) were determined after 72 h of incubation. The total number of *Mycobacterium* cells was calculated (2.97 × 10^8^ cells per ml at an OD500 of 0.10), and the cells were concentrated by centrifugation at 8,000 × g for 10 min. The cells were resuspended in Tris buffer (0.05 M, pH 7.5) at a concentration of 3 × 10^9/ml. Microcosms were inoculated with *Mycobacterium* cells at doses of either 1.5 × 10^5^ or 3.0 × 10^5^/g of moist sediment.

Autoclaved microcosms and microcosms lacking the *Mycobacterium* cells were included to detect abiotic and indigenous biotic PAH degradation. The number of *Mycobacterium* cells remaining in the inoculated microcosms after 10 weeks was determined by serial dilution of sediments and plating onto replicate MBS agar plates (*n* = 3 for each dilution) sprayed with pyrene by a method modified from that of Kiyohara et al. (13), as described previously (5). In addition, the *Mycobacterium* cells were inoculated into sterile microcosms to determine whether pyrene mineralization was enhanced or inhibited by microbial competition due to the presence of natural microbial populations.

**Mineralization of PAHs.** The rate of ^14^CO2, evolution resulting from microbial degradation of 2-methylnaphthalene, phenanthrene, pyrene, and benzo[a]pyrene was examined in replicate microcosms by methods described previously (4). Each microcosm was dosed with 0.5 μg of nonlabeled PAH per g and 0.92 μCi of ^14^C-labeled PAH (109 μg of PAH per microcosm) and incubated at 22°C in a temperature-controlled model 1210 Queue Series Mini-Room (Queue Systems, Parkersburg, W.Va.). Since surficial sediments are not static in natural ecosystems, the microcosms were mixed twice weekly by swirling the mixtures in tanks. Mineralization was measured in 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28 days. In addition, the degradation of each PAH as a component of a PAH mixture was examined. In this test series, each PAH (0.92 μCi of labeled PAH and 0.5 μg of unlabeled PAH per g) was introduced into replicate microcosms (*n* = 2) alone or with the three other unlabeled PAHs (0.5 μg/g) and incubated for 28 days at 24°C.

**Organic and inorganic nutrient enrichment.** Since high-molecular-weight PAHs are degraded by cometabolic reactions and inorganic nutrients may be limiting in some ecosystems, pyrene mineralization by the *Mycobacterium* sp. was measured in microcosms enriched with organic or inorganic nutrients. Organic enrichment of sediments was done with glucose and peptone at concentrations of 0.01% for each. Inorganic enrichment was done with 7.5 mg of KNO3 per liter for inorganic nitrogen and 0.5 mg of KH2PO4 per liter for inorganic phosphorus, which resulted in an N:P ratio of 15:1. Similar organic and inorganic enrichments have been used previously to examine the effects of nutrient enrichment on the biodegradation of phththalic acid esters in freshwater sediments (11).

**Effect on microcosms of preexposure to pyrene and preincubation with the Mycobacterium sp.** The survival of the *Mycobacterium* sp. in mixed microbial populations which occur naturally in ecosystems was determined by measuring pyrene mineralization in sediment-water microcosms after 6 weeks of preincubation with the *Mycobacterium* cells in the presence and absence of pyrene. Preexposure microcosms received 100 μg of nonlabeled pyrene per liter. Microcosms preincubated with the *Mycobacterium* sp. were inoculated with 1.5 × 10^5^ cells per g of moist sediment, as described above. Pyrene mineralization rates were compared with those of identical, control microcosms, which had been preincubated for 6 weeks with exposure to pyrene but which had not been inoculated with the *Mycobacterium* sp.

**RESULTS**

Enhanced mineralization of PAHs in microcosms. The PAHs selected for this experiment were representative of PAHs containing two (2-methylnaphthalene), three (phenanthrene), four (pyrene), and five (benzo[a]pyrene) fused aromatic rings. The mineralization of all four PAHs in microcosms from DeGray Reservoir was greatly enhanced by the presence of the *Mycobacterium* sp. (Fig. 1). For example, we previously reported (4) that 2-methylnaphthalene and phenanthrene were mineralized 10 and 14%, respectively, after 28 days in microcosms containing sediment and water from DeGray Reservoir (Fig. 1A). Mineralization of these two PAHs in the present study totaled 26 and 71%, respectively, after 28 days of incubation in similar microcosms inoculated with the *Mycobacterium* sp. Furthermore, we did not previously detect mineralization of pyrene or benzo[a]pyrene in microcosms from DeGray Reservoir, but in the present study mineralization totaled 40 and 3.6%, respectively, after 28 days in similar microcosms containing the *Mycobacterium* sp. (Fig. 1B). The inability of sediment and water from DeGray Reservoir to degrade these PAHs containing more than three aromatic rings was confirmed in the present study with control microcosms lacking the *Mycobacterium* sp. (Table 1).

Since PAHs usually occur concomitantly in the environment, the ability of the *Mycobacterium* sp. to enhance...
degradation of four different ring classes of PAHs as components in a mixture of four PAHs was evaluated in microcosms containing sediment and water from DeGray Reservoir (Fig. 2). Comparison of these data with those in Fig. 1B shows that mineralization of 2-methylnaphthalene and phenanthrene appears to be enhanced equally in the presence or absence of the other PAHs. However, pyrene mineralization was slightly lower in microcosms exposed to a mixture of PAHs. In contrast, these data suggest that benzo[a]pyrene was mineralized slightly faster in microcosms exposed to a mixture of PAHs.

The *Mycobacterium* sp. used in this study was originally isolated on pyrene-supplemented medium (7) and is known to selectively degrade some four-ringed PAHs faster than it does two- or three-ringed PAHs (6). Since pyrene mineralization does not usually occur in sediments and water from DeGray Reservoir (Fig. 1A) (2) but was detected at significant levels in the present study with microcosms containing the *Mycobacterium* cells (Fig. 1B), pyrene was selected as the sole PAH substrate in the following series of experiments. These subsequent studies were designed to determine the effects of microbial competition, inoculum size, PAH preexposure, and nutrient levels on the survival and function of the PAH-degrading *Mycobacterium* sp. in pristine sediment and water.

**Effects of microbial competition and inoculum size on pyrene mineralization.** Figure 3 shows pyrene mineralization in natural and sterilized microcosms from DeGray Reservoir that received a single inoculum of the PAH-degrading *Mycobacterium* sp. Pyrene mineralization was similar in both treatment groups and totaled about 40% after 3 weeks, with no lag phase. However, pyrene mineralization slowed in all microcosms after 3 weeks. Since a plateau in pyrene mineralization was observed in the microcosms, they were respiked with labeled and nonlabeled pyrene to determine whether the inoculated *Mycobacterium* sp. remained viable and functional after 6 weeks. The arrow in Fig. 3 indicates the time point for respiking. In both treatment groups, pyrene mineralization showed no lag phase after respiking and equaled or exceeded the total pyrene mineralization.

### Table 1. Effects of preincubation with a *Mycobacterium* sp. and preexposure to pyrene on the total percent of pyrene mineralized in microcosms from DeGray Reservoir

<table>
<thead>
<tr>
<th>Microcosm treatment</th>
<th>Total % of pyrene mineralized* on day:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6-wk preexposure to pyrene</td>
<td></td>
</tr>
<tr>
<td>Single <em>Mycobacterium</em> inoculum added at day 0</td>
<td>ND</td>
</tr>
<tr>
<td>Double <em>Mycobacterium</em> inoculum added at day 0</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>6-wk preincubation with a single <em>Mycobacterium</em> inoculum</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>6-wk preincubation with a single <em>Mycobacterium</em> inoculum + 6-wk preexposure to pyrene</td>
<td>2.5 ± 0.4</td>
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<tr>
<td>6-wk preincubation with a single <em>Mycobacterium</em> inoculum + 6-wk preexposure to pyrene</td>
<td>3.0 ± 0.1</td>
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*Values presented as total percent mineralized in replicate microcosms ± standard deviation. ND, not detected.

* Single and double *Mycobacterium* inoculum levels were 1.5 × 10⁵ and 3.0 × 10⁵ cells per g of sediment, respectively.

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FIG. 2. Mineralization of 2-methylnaphthalene (○), phenanthrene (●), pyrene (■), and benzo[a]pyrene (●) as components in a mixture of PAHs in microcosms from DeGray Reservoir.

FIG. 3. Pyrene mineralization in DeGray Reservoir microcosms which received a single inoculum of the *Mycobacterium* sp. (●) or a single inoculum of it after sterilization of the sediment and water (▲). The arrow indicates when microcosms were respiked with pyrene.
observed during the first 6 weeks of the experiment. These data indicate that the introduced Mycobacterium cells remained viable. The plateau observed in the mineralization of pyrene may result from sorption of the PAH onto the sediment matrix or may be related to the kinetics of degradation of pyrene by the microorganisms. We previously reported that most of the organic-extractable residues produced by this Mycobacterium sp. were metabolites resulting from the partial degradation of pyrene (5). Chemical analysis of organic-extractable residues in the microcosm water by previously reported methods (7) showed that, on chromatography, most of the 14C-residues remaining in the microcosms in the present study behaved in a manner similar to that of 4-phenanthroic acid, 4-hydroxyperinaphthenone, and other more highly polar metabolites (data not shown). These are the chemicals we previously reported to be polar metabolites of pyrene produced in pure culture by the Mycobacterium sp. (7).

Microbiological examination of respiked sediments at the end of the experiment resulted in the isolation of 2.8 × 10^6 Mycobacterium cells per g (wet weight) of sediment, which suggests an increase in the Mycobacterium population during the 10-week experiment. Since an increase in Mycobacterium populations was observed in the respiking experiments, we examined the effects of doubling the initial inoculum on the rate of pyrene mineralization in similar microcosms (Table 1). Doubling the Mycobacterium cell concentration increased pyrene mineralization only marginally after 28 days.

**Effects of preincubation with the Mycobacterium sp. and pyrene preexposure on pyrene mineralization.** Table 1 shows the effects of 6 weeks of preincubation with the Mycobacterium sp. and 6 weeks of preexposure to pyrene, alone and in combination, on pyrene mineralization in microcosms from DeGray Reservoir. Microcosms preincubated for 6 weeks with the Mycobacterium sp. mineralized pyrene with no lag phase, but the total amount of pyrene mineralized was similar to that observed in microcosms inoculated with the Mycobacterium sp. at day 0. In contrast, microcosms preincubated for 6 weeks with the Mycobacterium sp. and also preexposed for 6 weeks to pyrene showed slightly elevated levels of pyrene mineralization early in the experiment compared with microcosms inoculated with the Mycobacterium sp. at day 0. However, total pyrene mineralization in all microcosms containing the Mycobacterium sp. plateaued around 35 to 40% after 3 weeks, which is similar to the pattern observed in the experiments shown in Fig. 1, 2, and 3. No pyrene mineralization was detected in microcosms from DeGray Reservoir which had been preexposed to pyrene for 6 weeks but which had not been inoculated with the Mycobacterium sp.

**Effects of nutrient enrichment on pyrene mineralization.** Experiments were conducted to determine whether organic or inorganic nutrient enrichment would further enhance pyrene mineralization by the Mycobacterium sp. in microcosms from DeGray Reservoir (Fig. 4). The addition of organic nutrients resulted in decreased pyrene mineralization throughout the experiment. Turbidity and flocculant microbial growth were observed in all organic-nutrient-enriched microcosms. Direct examination of water samples from these microcosms by light microscopy showed that this biological material resulted from the growth of a diverse mixture of gram-negative and gram-positive bacteria and that it did not result solely from stimulated growth of the inoculant Mycobacterium sp. Pyrene mineralization in identical microcosms which received organic nutrients and twice the cell concentration of the Mycobacterium sp. was higher than that in microcosms which received a single Mycobacterium inoculum but was lower than that in control microcosms which received no nutrient addition.

Inorganic nutrient enrichment affected pyrene mineralization only minimally in microcosms throughout the study (Fig. 4). The level of pyrene mineralization in sterilized microcosms inoculated with the Mycobacterium sp. and supplemented with inorganic nutrients was similar to that in nonsterilized microcosms containing the Mycobacterium sp. and inorganic nutrients.

**DISCUSSION.**

The use of microorganisms for in situ removal of chemicals from the environment is dependent upon their survival and performance in natural ecosystems. Once a chemical-degrading microorganism is isolated or developed in the laboratory, it must undergo a series of experiments to evaluate its survival and performance among natural mixed microbial populations. In addition, it is useful to conduct studies to evaluate factors which may enhance or inhibit the performance of the chemical-degrading microorganism in the environment. Although a microorganism may perform well as a pure culture in the laboratory under optimal conditions, its performance may differ in natural ecosystems when the microorganism is subjected to various chemical and physical conditions and forced to compete with existing microbial populations. In this study, we report the use of a recently discovered PAH-degrading Mycobacterium sp. to enhance the degradation of four ring classes of PAHs in samples from a pristine ecosystem. In addition, we present the results from a series of experiments designed to evaluate the effects
of microbial competition, inoculum size, PAH preexposure, and nutrient enrichment on the performance of the PAH-degrading Mycobacterium sp. in sediment and water microcosms.

The PAH-degrading Mycobacterium sp. used in this study was recently isolated from an estuarine ecosystem near Port Aransas, Tex. (4). This ecosystem has been chronically exposed to petrogenic hydrocarbons from inland-oil-production and -storage facilities. We previously reported that sediments from this ecosystem had relatively high rates of PAH mineralization and elevated populations of hydrocarbon-degrading microorganisms (4). To date, the Mycobacterium sp. isolated from these sediments has been shown to mineralize naphthalene, phenanthrene, pyrene, fluoranthene, 1-nitropyrene, 6-nitrochrysene, and 3-methylcholanthrene in pure culture (5).

In the present study, the Mycobacterium sp. enhanced the degradation of four different ring classes of PAHs in microcosms containing sediment and water from DeGray Reservoir, a pristine ecosystem which has low levels of hydrocarbon-degrading bacteria, has low mineralization rates for two- and three-ringed PAHs, and is unable to degrade PAHs containing four or more fused aromatic rings (4). The inability of sediment and water from this ecosystem to degrade higher-molecular-weight PAHs was confirmed in the present study with controls lacking the Mycobacterium sp. It is noteworthy that although this Mycobacterium sp. is unable to mineralize benzo[a]pyrene in pure culture, the chemical analyses we previously reported for pure culture exposures showed that after 2 weeks of incubation 24.7% of the organic-extractable residues were metabolites which, on chromatography, behaved in a manner similar to that of possible ring oxidation products (5). The significant benzo[a]pyrene mineralization observed in DeGray Reservoir samples in the present study in microcosms inoculated with the Mycobacterium sp. supports our earlier conclusion that the production of degradable PAH metabolites by this bacterium may be an important factor in its ability to enhance the degradation of PAHs in natural environments.

We previously reported that in pure culture, the Mycobacterium sp. mineralized pyrene, a four-ringed PAH, faster than it degraded naphthalene, a two-ringed PAH, or phenanthrene, a three-ringed PAH (5). However, sediment and water microcosms inoculated with the microorganism in the present study mineralized phenanthrene faster than they mineralized pyrene (Fig. 1B and 2). It should be noted that mineralization of PAHs in microcosms in this study represents the sum of mineralization by both the Mycobacterium sp. and indigenous bacteria. Since the indigenous bacteria are able to mineralize phenanthrene but not pyrene, they can contribute directly to the total amount of phenanthrene mineralized but can only indirectly affect total pyrene mineralization by degrading metabolites produced by the Mycobacterium sp. In this case, pyrene mineralization in the microcosms would be dependent upon the rate of primary degradation by the Mycobacterium sp., whereas phenanthrene mineralization could be accomplished independently by the Mycobacterium sp. and the indigenous bacteria. This may explain why phenanthrene was mineralized faster than pyrene in microcosms in the present study and warrants further investigation.

PAHs commonly occur as diverse chemical mixtures in the environment (8, 10, 12). The PAH components of these mixtures may differ in molecular weight, number of aromatic rings, alkylation, and halogenation. In theory, the combined effects of these PAHs may be deleterious due to additive toxicity or may be beneficial due to higher total levels of hydrocarbon substrate for microbial growth and possible enhanced incineration of certain PAHs. The extent of mineralization observed for each of the PAHs tested in this study was not affected by coexposure with other PAHs, although marginally increased mineralization of benzo[a]pyrene and marginally decreased mineralization of pyrene in a mixture of PAHs were observed early in the study.

In a series of experiments utilizing pyrene as a sole PAH substrate, this study shows that the Mycobacterium sp. survived in microcosms from DeGray Reservoir, in the presence or absence of pyrene, and significantly mineralized multiple doses of pyrene. Since doubling the Mycobacterium inoculum size increased pyrene degradation only slightly, the optimal inoculum size for the Mycobacterium sp. in these sediments was ca. 1.5 × 10⁶ cells per g (wet weight). Competition with indigenous microorganisms did not inhibit the pyrene-degrading activity of the Mycobacterium sp. since similar rates of pyrene mineralization were observed for the microorganism in nonsterile and sterile microcosms. To date, several laboratory studies have monitored the survival of genetically engineered microorganisms in the environment (for a review, see reference 20). Although DNA-DNA colony hybridization methods have been used to track genotypes of bacteria catabolic for lower-molecular-weight PAHs (18), the present study is the first study in which a natural PAH-degrading microorganism has been shown to survive and to enhance the degradation of high-molecular-weight PAHs in sediment.

Since we previously reported that this microorganism did not utilize pyrene as a sole source of carbon and energy but that it apparently required an additional nutrient source, the mineralization of pyrene appears to occur by cometabolism (5, 6). However, organic nutrient enrichment inhibited pyrene degradation in the present study. This probably resulted from rapid utilization of peptone and glucose by rapidly growing, indigenous microorganisms instead of the inoculant Mycobacterium sp. This conclusion is supported by the occurrence of turbidity and flocculent microbial growth in organic-enriched microcosms which appeared to be a diverse mixture of bacteria when examined by light microscopy. Organic nutrient enrichment with identical concentrations of peptone and glucose has been reported to inhibit the degradation of di-n-butylphthalate (DBP) in freshwater sediments (11). This same previous study also reported that inorganic nutrient enrichment had no effect on DBP degradation rates. Similarly, identical inorganic nutrient enrichment in the present study had little effect on pyrene degradation in microcosms. The lack of further enhancement of pyrene degradation by nutrient enrichment in the present study should be regarded as a beneficial characteristic of the Mycobacterium sp. For example, nutrient enrichment of treatment sites for PAH degradation would add to the cost of bioremediation and could significantly alter the limnological characteristics of the ecosystem as a result of eutrophication.

Overall, this study demonstrates the versatility of this PAH-degrading Mycobacterium sp. and expands its potential applications to include the degradation of two-, three-, four-, and five-ringed PAHs in sediments. The Mycobacterium sp. survived and performed well in mixed sediment microbial populations and showed no need for organic or inorganic nutrient enrichment. Future laboratory studies in which the Mycobacterium sp. is inoculated into sediments highly contaminated with PAHs would be useful to fully
evaluate its performance in polluted ecosystems. In addition, since we previously determined that this microorganism grows well at salt concentrations of 3% (6), its potential for use for the bioremediation of PAHs in marine beaches or sediments warrants investigation.

LITERATURE CITED


