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MINIREVIEW

Biodegradation of High-Molecular-Weight Polycyclic Aromatic Hydrocarbons by Bacteria

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Interest in the biodegradation mechanisms and environmental fate of polycyclic aromatic hydrocarbons (PAHs) is prompted by their ubiquitous distribution and their potentially deleterious effects on human health. PAHs constitute a large and diverse class of organic compounds and are generally described as molecules which consist of three or more fused aromatic rings in various structural configurations (5). The biodegradation of PAHs by microorganisms is the subject of many excellent reviews (references 16, 17, 18, 30, 88, and 92, for example), and the biodegradation of PAHs composed of three rings is well documented. In the last decade, research pertaining specifically to the bacterial biodegradation of PAHs composed of more than three rings has been advanced significantly. The bacterial biodegradation of PAHs with more than three rings, which are often referred to in the biodegradation literature as high-molecular-weight (HMW) PAHs, is the subject of this minireview.

PAHs IN THE ENVIRONMENT

The chemical properties, and hence the environmental fate, of a PAH molecule are dependent in part upon both molecular size, i.e., the number of aromatic rings, and molecule topology or the pattern of ring linkage. Ring linkage patterns in PAHs may occur such that the tertiary carbon atoms are centers of two or three interlinked rings, as in the linear kata-annelated PAH anthracene or the pericondensed PAH pyrene. However, most PAHs occur as hybrids encompassing various structural components, such as in the PAH benzo[*a*]pyrene (B*a*P) (Fig. 1). Generally, an increase in the size and angularity of a PAH molecule results in a concomitant increase in hydrophobicity and electrochemical stability (39, 117). PAH molecule stability and hydrophobicity are two primary factors which contribute to the persistence of HMW PAHs in the environment.

Due to their lipophilic nature, PAHs have a high potential for biomagnification through trophic transfers (21, 69, 98). PAHs are also known to exert acutely toxic effects and/or possess mutagenic, teratogenic, or carcinogenic properties (18, 48, 82). Some PAHs are classified as priority pollutants by the U.S. Environmental Protection Agency (57, 90), and B*a*P is included as 1 of 12 target compounds or groups defined in the Environmental Protection Agency's new strategy for controlling persistent, bioaccumulative, and toxic pollutants (86). In addition to increases in environmental persistence with increasing PAH molecule size, evidence suggests that in some cases, PAH genotoxicity also increases with size, up to at least four or five fused benzene rings (17). The relationship between PAH environmental persistence and increasing numbers of benzene rings is consistent with the results of various studies correlating environmental biodegradation rates and PAH molecule size (2, 8, 40, 45). For example, reported half-lives in soil and sediment of the three-ring phenanthrene molecule may range from 16 to 126 days while for the five-ring molecule B*a*P they may range from 229 to >1,400 days (88).

PAHs are present as natural constituents in fossil fuels, are formed during the incomplete combustion of organic material, and are therefore present in relatively high concentrations in products of fossil fuel refining (7, 24, 66, 77, 78, 105, 106). Petroleum refining and transport activities are major contributors to localized loadings of PAHs into the environment. Such loadings may occur through discharge of industrial effluents and through accidental release of raw and refined products. However, PAHs released into the environment may originate from many sources, including gasoline and diesel fuel combustion (68, 71) and tobacco smoke (35), for example. PAHs are detected in air (63, 68), soil and sediment (47, 64, 65, 79, 99, 118), surface water, groundwater, and road runoff (11, 46, 72, 83); are dispersed from the atmosphere to vegetation (101); and contaminate foods (25, 67, 89). Anthropogenic and natural sources of PAHs in combination with global transport phenomena result in their worldwide distribution. Hence, the need to develop practical bioremediation strategies for heavily impacted sites is evident (38). PAH concentrations in the environment vary widely, depending on the proximity of the contaminated site to the production source, the level of industrial development, and the mode(s) of PAH transport. Soil and sediment PAH concentrations at contaminated and uncontaminated sites ranging from 1 μg/kg to over 300 g/kg have been reported (5, 51, 80, 84, 115).

HMW PAH BIODEGRADATION

The biochemical pathways for the biodegradation of aromatic compounds have been well described (30). It is understood that the initial step in the aerobic catabolism of a PAH molecule by bacteria occurs via oxidation of the PAH to a dihydrodiol by a multicomponent enzyme system. These dihydroxylated intermediates may then be processed through either an *ortho* cleavage type of pathway or a *meta* cleavage type of pathway, leading to central intermediates such as protocatechuates and catechols, which are further converted to tricarboxylic acid cycle intermediates (100). Amid early reports which described the microbial oxidation of HMW PAHs (30), Gibson et al. in 1975 (31) showed that treatment of a *Beijerinckia* sp. with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine created

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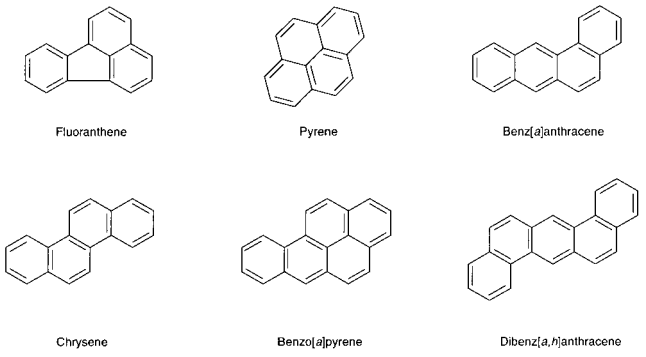

FIG. 1. Representative HMW PAHs.

a mutant (strain B8/36) which oxidized B*a*P and benz[*a*]anthracene to dihydrodiols after growth with succinate plus biphenyl. Two products of B*a*P metabolism were identified as *cis*-9,10-dihydroxy-9,10-dihydrobenzo[*a*]pyrene and *cis*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene. The main metabolites isolated from benz[*a*]anthracene metabolism were identified as *cis*-1,2-dihydroxy-1,2-dihydrobenz[*a*]anthracene and *cis*-8,9- and *cis*-10,11-dihydrodiols (31, 49). The cometabolic biodegradation of fluoranthene and B*a*P was also reported in 1975 (4). In the case of fluoranthene, concentrations near aqueous solubility were shown to be degraded by stationary-phase cultures of *Pseudomonas* strain NCIB 9816 grown on succinate and salicylate.

However, it was not until the late 1980s that three milestones in the biodegradation of HMW PAHs were reached. In 1988, Heitkamp and Cerniglia (41) published the first study on the isolation of a bacterium from the environment that could extensively degrade PAHs containing four aromatic rings. They described the isolation of a gram-positive rod from sediment near an oil field which cometabolically degraded a number of HMW PAHs (0.5 mg/liter), including fluoranthene, pyrene, 1-nitropyrene, 3-methylcholanthrene, 6-nitrochrysene, and B*a*P, when grown for 2 weeks with organic nutrients. Also in 1988, Mahaffey et al. (70) presented the first direct demonstration of ring fission during HMW PAH biodegradation. Following induction with biphenyl, *m*-xylene, and salicylate, *Beijerinckia* sp. strain B1 (reclassified as *Sphingomonas yanoikuyae* [62]) oxidized benz[*a*]anthracene to three *o*-hydroxypolyaromatic acids which were identified by nuclear magnetic resonance (NMR) and mass spectral analyses to be 1-hydroxy-2-anthranoic acid, 2-hydroxy-3-phenanthroic acid, and 3-hydroxy-2-phenanthroic acid. Mineralization experiments with [^{14}C]benz[*a*]anthracene also indicated the formation of $^{14}CO_2$. Lastly, Mueller et al. (75) in 1989 demonstrated for the first time that the utilization of a PAH containing four or more aromatic rings as a sole source of carbon and energy by bacteria is possible. They showed that a seven-member bacterial community isolated from creosote-contaminated soil was capable of utilizing fluoranthene. In addition, the community was capable of biotransforming other HMW PAHs in a concentration range of 0.3 to 2.3 mg/liter when grown on fluoranthene. During the ensuing decade, a diverse number of observations regarding the biodegradation of HMW PAHs by bacteria were published.

Four-ring PAHs. Of the four-ring PAHs, fluoranthene, pyrene, chrysene, and benz[*a*]anthracene have been investigated to various degrees in the biodegradation literature. Fluoranthene, a nonalternant PAH containing a five-membered ring, has been shown to be metabolized by a variety of bacteria, and pathways describing its biodegradation have been proposed. In 1990, two reports documenting the isolation of a single organism capable of utilizing fluoranthene as a sole source of carbon and energy were published by independent research groups. Weissenfels et al. (107) documented the isolation of the soil microorganism *Alcaligenes denitrificans* strain WW1, which biodegraded fluoranthene at a rate of 0.3 mg/ml per day and which also cometabolized other PAHs, including pyrene and benz[*a*]anthracene (108). Metabolite identification indicated biodegradation via a dioxygenase pathway. Three metabolites of fluoranthene biodegradation by strain WW1, 7-hydroxyacenaphthylene, 7-acenaphthenone, and 3-hydroxymethyl-4,5-benzocoumarine, were identified by UV, mass, and NMR spectroscopic methods, and a pathway was proposed (108; Fig. 2). Also in 1990, Mueller et al. (74) isolated the bacterium *Pseudomonas* (*Sphingomonas*) *paucimobilis* EPA505 from a seven-member bacterial community (75) which was capable of utilizing fluoranthene as a sole source of carbon and energy. Utilization of fluoranthene was demonstrated by increases in bacterial biomass, disappearance of fluoranthene from an aqueous solution, and transient production of fluoranthene metabolites. A resting cell suspension of EPA505 grown on fluoranthene or complex medium was also capable of biotransforming other four-ring and five-ring PAHs (74, 116). The four-ring compounds included benzo[*b*]fluorene, benz[*a*]anthracene, chrysene, and pyrene. Strain EPA505 cometabolically mineralized radiolabeled chrysene to nearly 42% $^{14}CO_2$ in 48 h, but no $^{14}CO_2$ was detected from cultures incubated with radiolabeled pyrene (116).

Fluoranthene metabolites resulting from degradation by a *Mycobacterium* species have also been reported. Kelley and Cerniglia (58) showed that in mineral medium supplemented with organic nutrients, *Mycobacterium* sp. strain PYR-1 was capable of degrading greater than 95% of added fluoranthene within 24 h. Fluoranthene concentrations as high as 17 mg/liter did not inhibit microbial growth, and when added to soil and water microcosms, the mycobacterium enhanced the mineralization of fluoranthene over that due to the indigenous biota. Although degradation of fluoranthene to CO_2 was rapid, a small amount of a ring cleavage metabolite was detected (0.65% of the total radioactivity added) and characterized as 9-fluorenone-1-carboxylic acid (60). Further investigations revealed the presence of 10 more fluoranthene metabolites which were identified as 8-hydroxy-7-methoxyfluoranthene, 9-hydroxyfluorene, 9-fluorenone, 1-acenaphthenone, 9-hydroxy-1-fluorenecarboxylic acid, phthalic acid, 2-carboxybenzaldehyde, benzoic acid, phenylacetic acid, and adipic acid. Authentic standards of 9-hydroxyfluorene and 9-fluorenone were also metabolized, and pathways were proposed for the metabolism of fluoranthene by *Mycobacterium* sp. strain PYR-1 (17, 61; Fig. 2). In a six-component synthetic mixture of three-, four-, and five-ring PAHs, *Mycobacterium* PYR-1 degraded all of the components to various degrees, with the exception of chrysene (59).

Fluoranthene has been used as a model compound in studies which have investigated the effects of surface-active compounds on PAH biodegradation. Comparisons of the mineralization of fluoranthene by four fluoranthene-degrading strains in the presence of the nonionic surfactants Triton X-100 and Tween 80 showed that responses differed between strains. The authors concluded that optimal conditions for PAH mineralization may be developed after assessment of degradation parameters for a particular strain (114). Triton X-100 was found to more than double the mineralization rate of fluoranthene by *S. paucimobilis* EPA505 when divalent cations such as calcium were present. Without calcium, Triton X-100 inhibited fluoranthene mineralization, possibly by adversely affecting the functioning of the cytoplasmic membrane (113). A model which described the degradation kinetics of Triton X-100-sol-

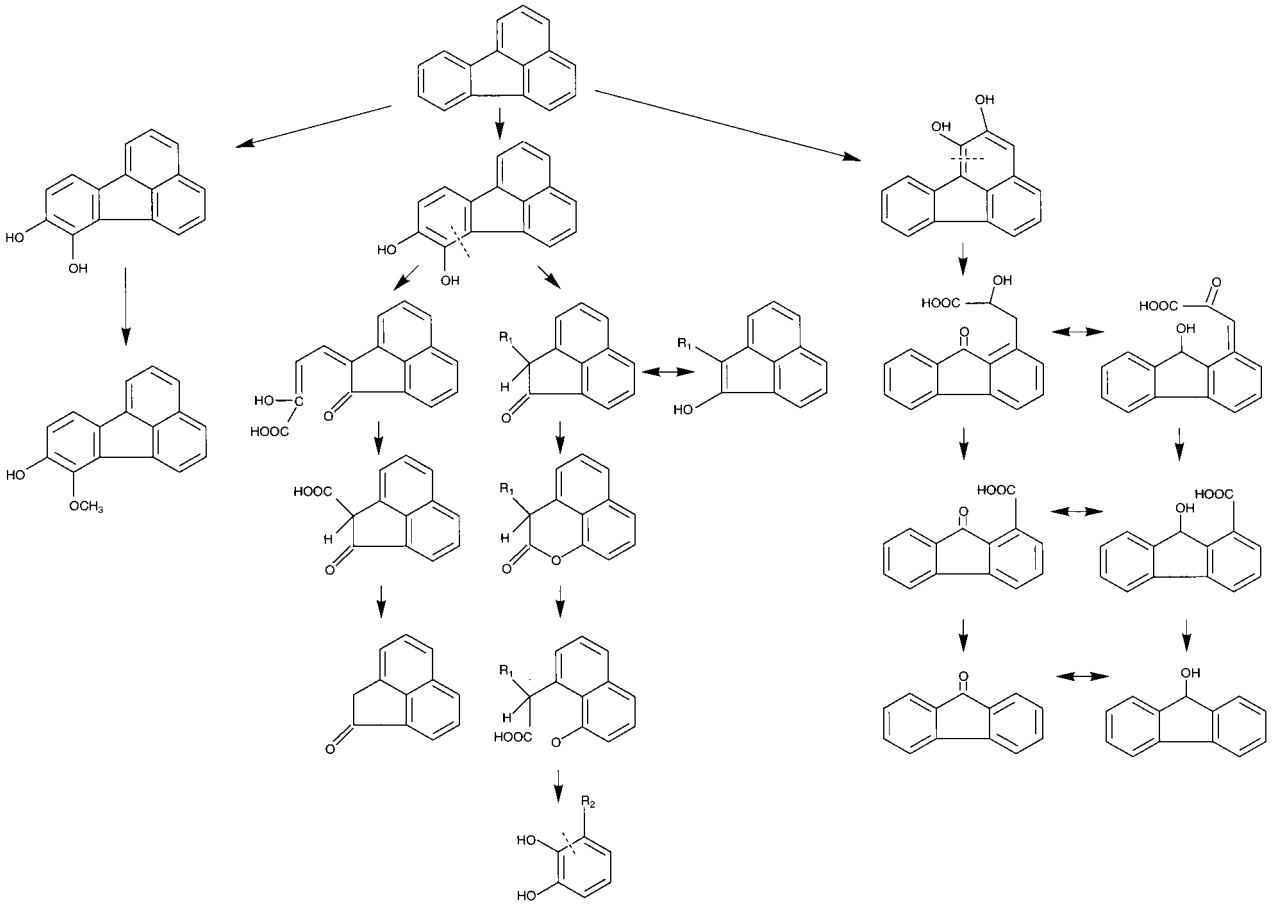

FIG. 2. Pathways proposed for the metabolism of fluoranthene by bacteria (17, 61, 108).

ubilized fluoranthene by *S. paucimobilis* EPA505 cells was also developed (111). By increasing the apparent aqueous solubility of fluoranthene, the bioemulsifier alasan was found to more than double the rate of [^{14}C]fluoranthene mineralization by *S. paucimobilis* EPA505 (3). Furthermore, fluoranthene-degrading organisms have been isolated from soil for biosurfactant and bioemulsifier screening purposes (112).

The bacterial degradation of pyrene, a pericondensed PAH, has been reported by a number of groups, and some have identified metabolites and proposed pathways. A *Mycobacterium* sp. isolated from sediment near a hydrocarbon source (19, 41, 43) mineralized pyrene when grown in mineral salts medium supplemented with organic nutrients. Pyrene-induced *Mycobacterium* cultures mineralized over 60% of radiolabeled pyrene in 96 h. Inducible enzymes appeared to be responsible for pyrene catabolism, since lag phases in pyrene mineralization were observed in cultures grown in the absence of pyrene and no pyrene mineralization was observed in noninduced cultures dosed with chloramphenicol, an inhibitor of bacterial protein synthesis (43). Seven metabolites of pyrene metabolism were detected by high-pressure liquid chromatography (44). Three products of ring oxidation, *cis*-4,5-pyrenedihydrodiol, *trans*-4,5-pyrenedihydrodiol, and pyrenol, and four products of ring fission, 4-hydroxyperinaphthenone, 4-phenanthroic acid, phthalic acid, and cinnamic acid, were identified by multiple analyses, including UV, infrared, mass, and NMR spectrometries and gas chromatography. 4-Phenanthroic acid was the major metabolite, and it was unclear if the small amounts of pyrenol detected had occurred by nonenzymatic dehydration of pyrene dihydrodiols or by oxidative metabolism of pyrene by the mycobacterium. Interestingly, the detection of both *cis*- and *trans*-4,5-dihydrodiols suggested multiple pathways for the initial oxidative attack on pyrene. Studies using $^{18}O_2$ confirmed that these products were indeed catalyzed by dioxygenase and monooxygenase enzymes, respectively. Sediment microcosms inoculated with the mycobacterium showed enhanced mineralization of various PAHs, including pyrene and B*a*P (42). Although the degradation of pyrene by this strain was cometabolic, increases in organic nutrients to the microcosm inhibited pyrene degradation. This most likely occurred due to the utilization of nutrients by rapidly growing, indigenous microorganisms instead of the inoculant *Mycobacterium* sp. Pathways documenting the biodegradation of pyrene were later proposed by Cerniglia (17; Fig. 3). Key elements of the proposed pathway were again confirmed after the identification of metabolites from the bacterial isolates *Mycobacterium* sp. strain RJGII-145 (see below), *Mycobacterium flavescens* (23), and *Mycobacterium* sp. strain KR2 (85). *M. flavescens* and strain KR2 were capable of growth on pyrene as a sole source of carbon and energy (23, 85).

In soils found to mineralize pyrene, Grosser et al. (34) isolated *Mycobacterium* sp. strain RJGII-135, which utilized pyrene as a sole source of carbon and energy. Enhanced mineralization of pyrene in soil was demonstrated after reintroduction of the organism into soil following growth in pure culture on pyrene. Pyrene mineralization reached 55% within

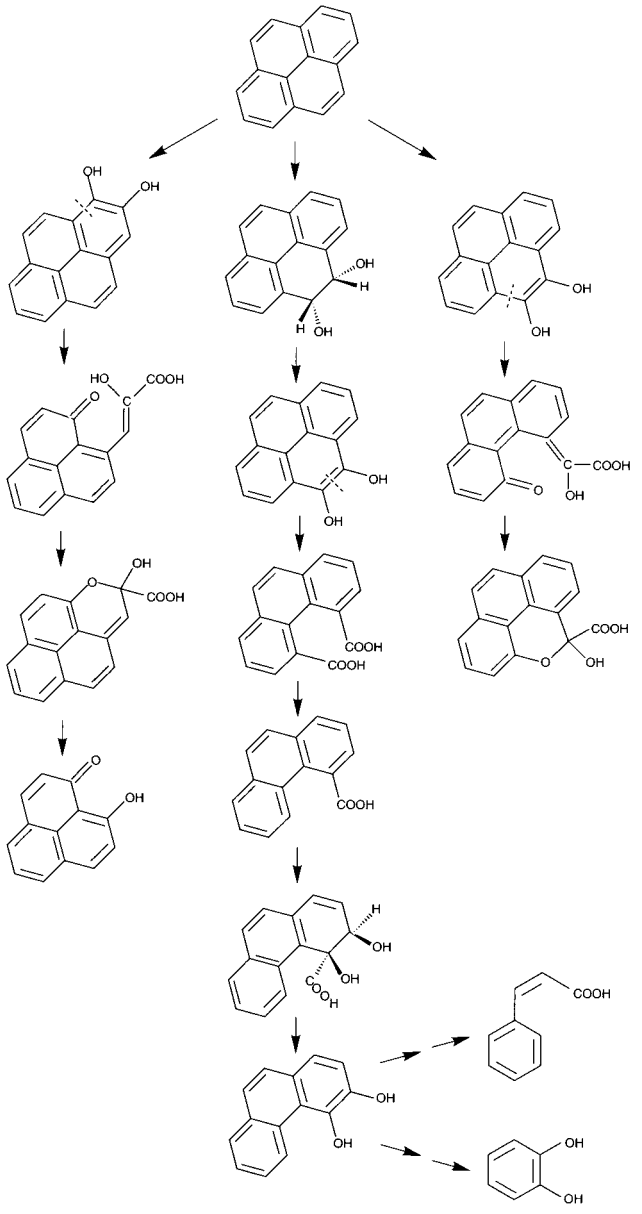

FIG. 3. Pathways proposed for the metabolism of pyrene by bacteria (17, 44, 102).

2 days, compared with a level of 1% for the indigenous population. Metabolites of pyrene degradation were identified in further degradation studies involving strain RJGII-135 (87). During mineralization of pyrene, three stable intermediates were formed within 4 to 8 h after the start of the experiment. Two of the intermediates, 4-phenanthrenecarboxylic acid and 4,5-pyrenedihydrodiol, were previously shown to be produced by *Mycobacterium* strain PYR-1 (44); however, the third metabolite, 4,5-phenanthrenedicarboxylic acid, had been proposed (17) but not isolated previously. Phylogenetic comparisons were made between *Mycobacterium* sp. strains PYR-1 and RJGII-135 (33, 103), and a quantitative method for detection of these two strains by PCR was developed to monitor cell concentrations during the bioremediation of PAH-contaminated soil (104). Strain RJGII-135 was also capable of cometabolic benz[*a*]anthracene metabolism and formed three dihydrodiols which included the newly identified 5,6-dihydrodiol (87) and the 10,11- and 8,9-dihydrodiols which were identified previously (31, 49, 70).

Mycobacterium sp. strain BB1 was isolated from a former coal gasification site and exhibited exponential growth in fermentor cultures when grown on solid fluoranthene (0.056 h^{-1}) and pyrene (0.04 h^{-1}) as sole sources of carbon and energy (6). Strain BB1 was used to examine the effects of various culture conditions on the biodegradation of PAHs, including the degradation of pyrene at low defined oxygen concentrations (27) and the utilization of PAHs in mixtures (95). In addition, nonionic surfactants which were not utilized preferentially as growth substrates and which were not toxic to strain BB1 were found to enhance the degradation of fluoranthene and pyrene (94). Also working with a *Mycobacterium* sp. soil isolate that utilized pyrene as a sole source of carbon and energy, Jimenez and Bartha (50) demonstrated solvent-augmented mineralization of pyrene. They showed that cells which physically adhered to solvent droplets containing pyrene in mineral medium were capable of mineralizing pyrene 8.5 times faster than suspended cells in the aqueous phase.

Recently, *Mycobacterium* sp. strain CH1, which was isolated from PAH-contaminated freshwater sediments and which mineralized fluoranthene and pyrene (pyrene as the sole carbon and energy source), was also capable of using a wide range of branched alkanes and *n*-alkanes as sole carbon and energy sources (20). The lack of hybridization of strain CH1 DNA with the *nahAc* gene showed that the enzyme system involved in PAH degradation is unrelated to the naphthalene dioxygenase pathway. Furthermore, weak hybridization of the *alkB* gene probe to strain CH1 DNA suggested only a limited homology between this strain and genes involved in *P. oleovorans* alkane oxidation. These new observations have led Churchill et al. to suggest that the occurrence of both aromatic and aliphatic hydrocarbon-degradative capacities within a single strain may be more common than was previously thought, as studies have recently emerged which address this phenomenon (91, 109).

Rhodococcus sp. strain UW1 isolated from contaminated soil was capable of utilizing pyrene and chrysene as sole sources of carbon and energy (102). A metabolite of pyrene degradation with the molecular formula $C_{16}H_{10}O_4$ was recovered. It was presumed to be the result of the recyclization of the direct *meta*-ring fission product of pyrene and because it was not clear whether ring cleavage occurred at the 1,2 or 4,5 positions, two pathways were proposed (Fig. 3). Bouchez et al. (9) used six bacterial strains, which included two *Rhodococcus* spp. capable of growth on pyrene and fluoranthene, to investigate the degradation of PAHs in binary mixtures. All individual strains were capable of cometabolic degradation of PAHs, and inhibition and synergistic interactions were observed. Inhibition was most commonly observed when the added PAH was more water soluble than the PAH added originally. It was also observed that mineralization yields were higher and biomass yields were lower for HMW PAH-degrading bacteria than for low-molecular-weight PAH-degrading bacteria (10).

In soil screenings for PAH-degrading bacteria, *Gordona* sp. strain BP9 (the genus *Gordona* was originally classified as part of the genus *Rhodococcus*) and *Mycobacterium* sp. strain VF1 were isolated from hydrocarbon-contaminated soil and each was capable of utilizing fluoranthene and pyrene as sole carbon and energy sources (55). Reintroduction of BP9 into soil after growth on 200 mg of pyrene per liter in a pure culture showed that a sixfold increase in pyrene metabolism was achieved compared to native uninoculated soil (56). Two pyrene-utiliz-

ing soil pseudomonads which were also capable of growing on fluoranthene were used in soil reinoculation experiments to test the effects of four surfactants on pyrene degradation (93). Soil inoculation of pyrene degraders in the presence of surfactant was shown to increase mineralization of pyrene under unsaturated conditions; however, pyrene degraders inoculated into soil without surfactant were more effective at degrading pyrene in soil slurries.

Although many of the HMW PAH-degrading bacteria described are actinomycetes, a variety of non-actinomycete bacteria have also been reported to metabolize fluoranthene, pyrene, chrysene, and benz[*a*]anthracene. *P. putida*, *P. aeruginosa*, *Flavobacterium* sp., and an unidentified strain were isolated from a soil-derived mixed culture which was capable of metabolizing fluoranthene and pyrene when supplemented with other forms of organic carbon (96). These strains, when recombined into a mixed culture, were found to degrade PAHs in a fashion similar to that of the original culture. *Cycloclasticus* strains isolated from marine sediments were capable of partially degrading 1 ppm pyrene or 1 ppm fluoranthene cometabolically when provided with 10 ppm phenanthrene (28). Three *Burkholderia cepacia* strains isolated from soil grew on pyrene at concentrations of up to 1,000 mg/liter and also degraded fluoranthene and benz[*a*]anthracene as sole carbon and energy sources (52). *S. yanoikuyae* (its history and aromatic hydrocarbon-degrading abilities were recently reviewed [29]) has been shown to oxidize chrysene (12), while *P. fluorescens* strain P2a utilized chrysene and benz[*a*]anthracene as sole carbon sources (13). Enzyme expression in strain P2a was further evaluated (15). Another *Pseudomonas* organism, strain HL7b, was isolated from enrichment cultures derived from the aromatic fraction of crude oil and was reported to degrade fluoranthene, but not as a sole carbon and energy source (26).

PAHs with more than four rings. Currently, there is only limited information regarding the bacterial biodegradation of PAHs with five or more rings in both environmental samples and pure or mixed cultures. Most studies have focused on the five-ring B*a*P molecule due its potential hazards to human health. B*a*P may be activated metabolically to a potent carcinogen, and extensive studies on the mechanism by which it and other PAHs induce neoplasia were initiated after the identification of B*a*P as a major active component of coal tar during the 1930s (82, 97). Although B*a*P is detected in a variety of environmental samples, the highest concentrations of B*a*P are often found in soils and sediments. Many studies have documented the environmental recalcitrance of B*a*P in these media (8, 17, 32, 81, 99, 110). Turnover times of greater than 3.3 years in oil-contaminated freshwater sediments and possibly greater than 60 years in uncontaminated sediments have been reported for the biotransformation of B*a*P, for example (45). In soils that readily accommodated the mineralization of other three- and four-ring PAHs, Carmichael and Pfaender (14) showed that only 2 to 9% of [^{14}C]B*a*P at 136 ng/g was mineralized in 8 weeks while in a soil from a previously contaminated coal gasification site, 25% of [^{14}C]B*a*P at 84 ng/g was mineralized to $^{14}CO_2$ during a 225-day incubation period (34). However, it has been shown recently that extensive cometabolic mineralization of [^{14}C]B*a*P may occur in soil at B*a*P concentrations ranging from 67 to 80 μg/g (53, 54). As shown in Fig. 4A, after an incubation period of 100 days, approximately 40% of [^{14}C]B*a*P was transformed into $^{14}CO_2$ when the indigenous soil microbiota was provided with a suitable cosubstrate (54).

B*a*P biodegradation by pure and mixed cultures of bacteria has been shown to occur, albeit bacteria capable of utilizing B*a*P as a sole source of carbon and energy have never been demonstrated. All reported B*a*P biotransformations by bacteria have therefore occurred under cometabolic conditions. As already mentioned, early observations of B*a*P biodegradation were made with mutant *Beijerinckia* sp. strain B8/36 grown on succinate plus biphenyl (31) and with *Pseudomonas* strain NCIB 9816 grown on succinate plus salicylate (4). When grown with peptone at 250 μg/liter, yeast extract, and soluble starch, *Mycobacterium* sp. strain PYR-1 biotransformed 0.5 mg of B*a*P per liter to 24.7% aqueous and organic-extractable metabolites even though no B*a*P mineralization was detected (41). Using the same mycobacterium, slight mineralization of B*a*P in sediment-water microcosms (180 ml of lake water plus 20 g [wet weight] of sediment) was demonstrated. The total mass of B*a*P added was 0.5 μg (25 ppb in sediment), and after 28 days, 3.6% of the total B*a*P added was mineralized to $^{14}CO_2$ (42). Strain PYR-1 was also shown to slightly degrade B*a*P in a six-component PAH mixture (59).

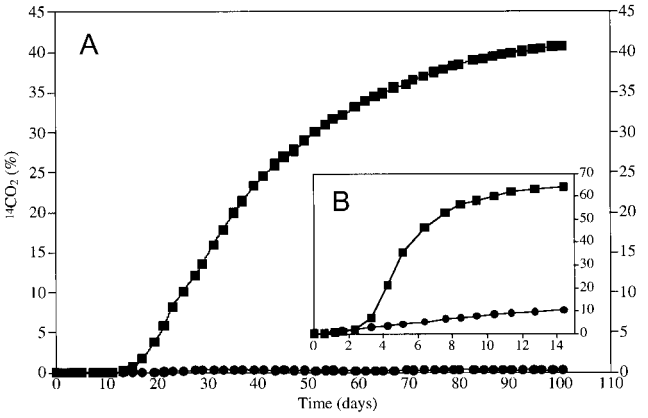

FIG. 4. Mineralization of [7-^{14}C]B*a*P in soil (80 μg/g) with (■) or without (●) 1.0% (wt/wt) crude oil (A) and in a liquid culture (10 mg/liter) obtained from the same soil with (■) or without (●) 0.2% (wt/wt) diesel fuel (B) (Kanaly and Bartha, 98th Gen. Meet. Am. Soc. Microbiol.) (Reprinted with permission from reference 54).

Supporting the dioxygenase enzymatic processes reported previously for other bacteria, Schneider et al. (87) published the only paper which describes the identification of B*a*P ring fission products, *Mycobacterium* sp. strain RJGII-135 grown on a mixture of yeast extract, peptone, and soluble starch was capable of biotransforming 20 μg of B*a*P in 50 ml. Metabolites of B*a*P biodegradation were detected, and as shown in Fig. 5, were identified by high-resolution mass spectrometry to be *cis*-7,8-benzo[*a*]pyrenedihydrodiol, 4,5-chrysenedicarboxylic acid, *cis*-4-(8-hydroxypyren-7-yl)-2-oxobut-3-enoic acid [or *cis*-4-(7-hydroxypren-8-yl)-2-oxobut-3-enoic acid], and 7,8-dihydropyrene-7-carboxylic acid (or 7,8-dihydropyrene-8-carboxylic acid). The authors were unable to distinguish between the *meta* fission products through the 7,8 bond and the 9,10 bond of B*a*P, hence the possibility of two products for two of the metabolites.

There are few studies which document extensive mineralization of PAHs with more than four rings. The PAH-degrading versatility of *S. paucimobilis* EPA505 was demonstrated by Ye et al. (116). After 16 h of incubation with 10 mg of an HMW PAH per liter, a resting cell suspension of EPA505 was capable of mineralizing the five-ring PAHs B*a*P, benzo[*b*]fluoranthene, and dibenz[*a,h*]anthracene to 33.3, 12.5, and 7.8% $^{14}CO_2$, respectively. No mineralization was detected for the six-ring PAH dibenz[*a,l*]pyrene. In conjunction with previous data (74), the authors reported that fluoranthene was capable of inducing the enzyme(s) necessary for the microbial degradation of a

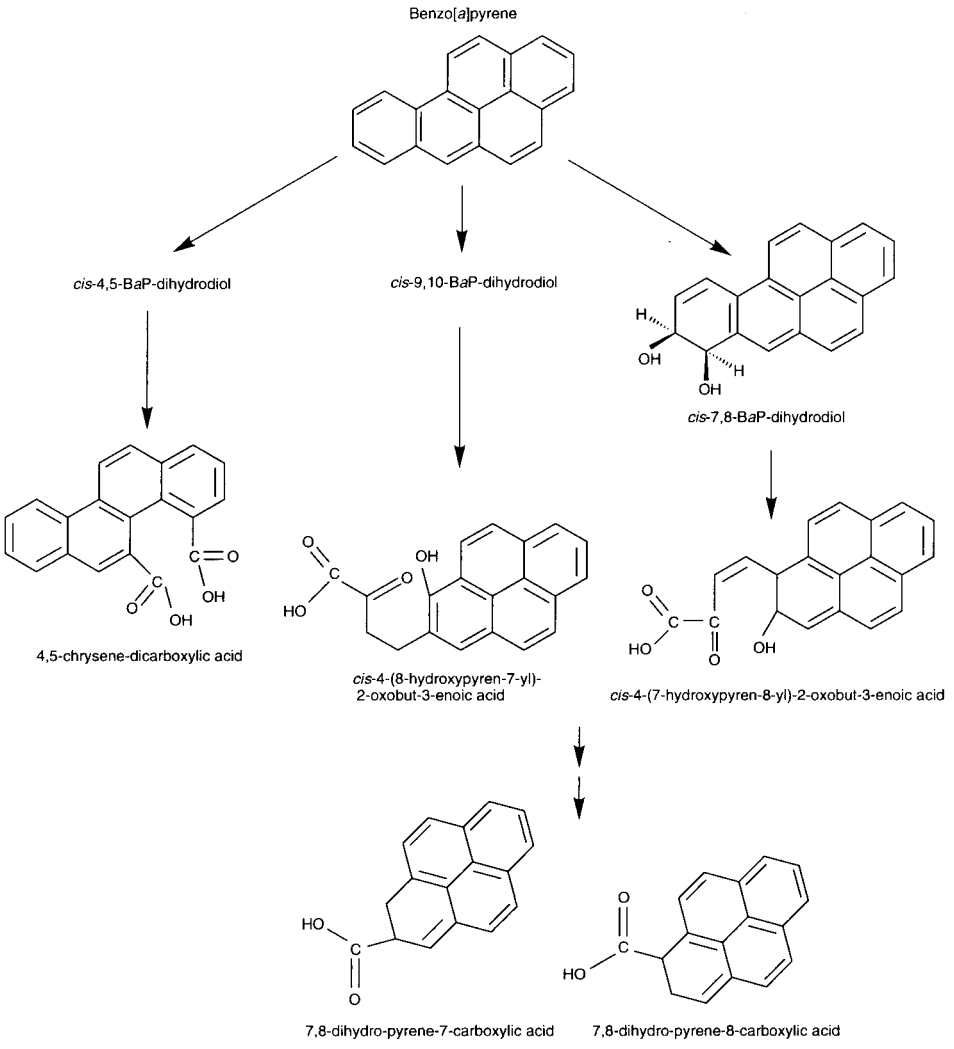

FIG. 5. Pathways proposed for the metabolism of B*a*P by *Mycobacterium* sp. strain RJGII-135 (87). Structures of identified metabolites are shown.

variety of PAHs. B*a*P biodegradation was not affected by the presence of co-occurring PAHs, except in the case of benzo[*b*]fluoranthene. These results suggested that the PAHs tested do not compete for the same active site or that the enzyme(s) responsible for the biodegradation of B*a*P is different from that of the other PAHs used in the study. Currently, we are investigating a bacterial consortium derived from soil (54) which cometabolically mineralizes [^{14}C]B*a*P to greater than 60% $^{14}CO_2$ in 2 weeks (Fig. 4B) when provided with a complex hydrocarbon cosubstrate such as diesel fuel (R. A. Kanaly and R. Bartha, Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. Q-283, p. 467, 1998; R. A. Kanaly, R. F. Sullivan, and R. Bartha, Program Abstr. 8th Int. Symp. Microb. Ecol., abstr. 55, p. 194, 1998).

Cometabolic biodegradation of dibenz[*a,h*]anthracene and B*a*P by *B. cepacia* strains was demonstrated when 100 mg of phenanthrene per liter was added to cultures containing 50 mg of either compound per liter (52). Decreases of 41 and 52% in dibenz[*a,h*]anthracene and B*a*P, respectively, were observed after 56 days. Furthermore, *B. cepacia* strain VUN 10003 degraded 11.65 mg of dibenz[*a,h*]anthracene per liter as a sole carbon and energy source after 56 days (52). In a mixed bacterial culture, approximately 2 mg of B*a*P per liter was reported to be degraded in 12 days in liquid medium supplemented with yeast extract at 2,000 mg/liter (96). Although progress has been made pertaining to the biodegradation of PAHs with more than four rings, most notably, the identification of B*a*P ring fission products (87), it is clear that there is a strong need for better understanding in this area.

In summary, knowledge regarding the bacterial biodegradation of HMW PAHs has been advanced in the last decade. A number of HMW PAH-degrading strains have been isolated and characterized. During the same period, the number of HMW PAH compounds known to be degraded by these isolates and consortia has also increased and pathways for the degradation of HMW PAHs by some strains have been elucidated. Still, further explorations are required in several areas of HMW PAH biodegradation research. Investigations into the regulatory mechanisms of HMW PAH biodegradation, the biodegradation of PAHs combined with other hydrocarbons in mixtures, and the microbial interactions within PAH-degrading consortia are examples of areas where research is needed. Fresh insights into HMW PAH biodegradation, such as the observation that fluoranthene is anaerobically oxidized to carbon dioxide under sulfate-reducing conditions in ocean sediments (22), show that knowledge in the field of PAH biodeg-

radation is expanding in new directions. Advances in molecular biology are aiding in the detection of PAH-degrading organisms from environmental samples (1, 37, 76) or in the differential detection of enzymes (73). Increases in our understanding of the microbial ecology of HMW PAH-degrading communities and the mechanisms by which HMW PAH biodegradation occur will prove helpful for predicting the environmental fate of these compounds and for developing practical PAH bioremediation strategies in the future.

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