Polycyclic aromatic hydrocarbon bioremediation design

Shigeaki Harayama

Many polycyclic aromatic hydrocarbons (PAHs) are known to be mutagenic or carcinogenic, and their contamination in soil and aquifer is of great environmental concern. Limited numbers of microorganisms including mycobacteria, *Sphingomonas* and white rot fungi were found to be capable of degrading PAHs with four or more fused aromatic rings. In white rot fungi, lignin peroxidases are believed to be involved in the degradation of PAHs. In addition to these enzymes, P450 monooxygenases in some fungi were implicated in the degradation of PAHs. The stimulation of PAH biodegradation by the addition of surfactants was observed with some of these microorganisms although the agents were inhibitory on biodegradation with some other microorganisms. Mathematical models were constructed to explain the effect of surfactants on biodegradation. Further studies should be carried out to select the best microorganisms and surfactants for applications to PAH bioremediation.

Addresses
Marine Biotechnology Institute, 3-75-1 Heita, Kamaishi, Iwate 026, Japan; e-mail: harayama@kamaishi.mbio.co.jp

Current Opinion in Biotechnology 1997, 8:268–273

http://biomednet.com/elecref/0958166900800268

Abbreviations

LiP	lignin peroxidase
MnP	manganese peroxidase
PAH	polycyclic aromatic hydrocarbon
RT-PCR	reverse transcriptase-PCR

Introduction

PAHs, which are compounds composed of two or more fused aromatic rings (Fig. 1), are widely distributed in the natural environment. Coal and petroleum are two major natural sources of PAHs. Contamination by PAHs at a high level is thus found at coal and petroleum treatment sites, including creosote wood treatment facilities (creosote is used as a lumber preservative and contains many PAHs). PAHs, particularly the higher molecular weight types, cause great environmental concern because of their mutagenic and carcinogenic properties [1,2]. Despite this, cleaning up PAH-contaminated sites using biological treatment has not been frequently applied [3]. There are two reasons for this relative lack of bioremediation of PAH-contaminated sites: first, although biological methods have been successfully used to treat municipal and industrial waste water, their application in land remediation is still in a stage of infancy: second, PAHs are refractory to biodegradation and persist in the natural environment because of their hydrophobic nature, resulting in low water solubility and a tendency to be adsorbed to the matrix of soil and sediment. The successful application of microorganisms to the bioremediation of PAH-contaminated sites thus requires a deeper understanding of how microbial biodegradation proceeds in PAHs. In this review, the bacteria involved and the metabolic pathways for the degradation of PAHs are summarized and the biological constraints on the PAH bioremediation are discussed.

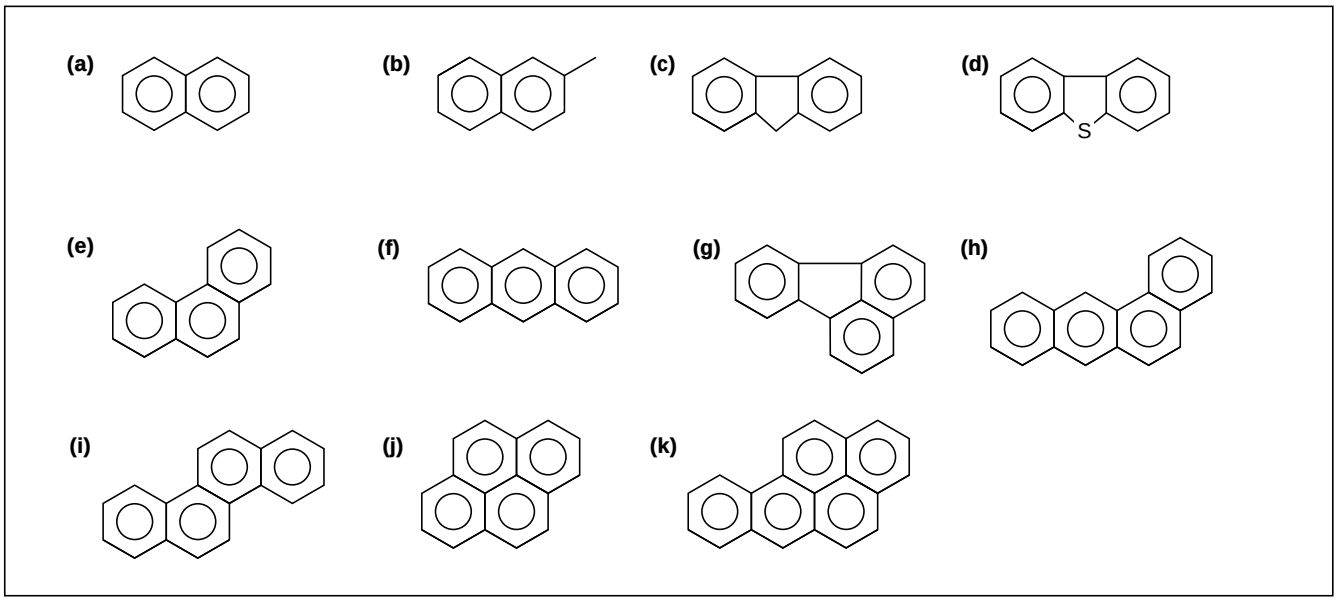
PAH-degrading bacteria

The biodegradation of PAHs has been observed under both aerobic and anaerobic conditions. The anaerobic biodegradation of PAHs is a slow process, and its biochemical mechanism has not yet been elucidated [4,5]. In contrast, aerobic biodegradative pathways, especially those for simple PAHs such as naphthalene and phenanthrene, have been extensively studied over the past decade. These pathways initiate the biodegradation of PAHs by introducing both atoms of molecular oxygen into the aromatic nucleus, the reaction being catalyzed by a multicomponent dioxygenase which consists of a reductase, a ferredoxin and an iron-sulfur protein [6]. Further reactions lead to the formation of precursors of tricarboxylic acid cycle intermediates. The genes for the initial steps in the degradation of phenanthrene, naphthalene and dibenzothiophene have been cloned and sequenced in many strains. The amino acid sequences of the catabolic enzymes deduced from their nucleotide sequences are 90% identical to each other. Less homologous groups of genes coding for enzymes involved in the degradation of PAHs have been found in *Comamonas testosteroni* [7] and *Nocardioides* sp. [8]. The pathway for the degradation of naphthalene generally exhibits broad substrate specificity; for example, *Burkholderia cepacia* F297 grows on a wide variety of polycyclic aromatic compounds including fluorene, (methyl)naphthalene, phenanthrene, anthracene and dibenzothiophene. An analysis of the intermediates formed from these growth substrates has indicated that these compounds are degraded by catalytic reactions very similar to those for naphthalene degradation [9].

A new fluorene catabolic pathway has recently been found in which hydroxylation at C-9 of fluorene generates 9-fluorenol, which is then dehydrogenated to 9-fluorenone [10]. This intermediate then undergoes dioxygenation at an angular site to form 1,10-dihydro-1,10-dihydroxyfluorene-9-one (DDF), the five-membered ring of which is subsequently cleaved to generate a substituted biphenyl. The ring-cleavage enzyme was a purified and characterized NAD^+-dependent DDF dehydrogenase (NAD, nicotinamide adenine dinucleotide).

A particular pathway for the degradation of dibenzothiophene has also been described [11]. This pathway converts dibenzothiophene to 2-hydroxybiphenyl via dibenzothio-

Figure 1



Structures of representative PAHs. **(a)** Naphthalene. **(b)** 2-methylnaphthalene. **(c)** Fluorene. **(d)** Dibenzothiophene. **(e)** Phenanthrene. **(f)** Anthracene. **(g)** Fluoranthene. **(h)** Benz[*a*]anthracene. **(i)** Chrysene. **(j)** Pyrene. **(k)** Benzo[*a*]pyrene.

phene sulfone: the genes necessary for this conversion have been cloned and sequenced. This catabolic route is believed to be useful for the desulfurization of sulfur-containing fossil fuel [12,13].

Although the catabolism of PAHs possessing three or fewer fused aromatic rings has been well studied, the biodegradation of the larger PAHs has not been studied as extensively. Interestingly, mycobacteria have been repeatedly isolated as bacteria that are able to degrade PAHs possessing four or more fused aromatic rings. The hydrophobic cell surface may be advantageous for their adhesion to insoluble PAHs, thus facilitating mass transfer of the substrates inside the cells. A *Mycobacterium* strain, PYR-I, has been found to mineralize pyrene and fluoranthene more rapidly than naphthalene and phenanthrene [14]. This strain can transform but not mineralize benzo[*a*]pyrene. Another *Mycobacterium* strain, RJGII-135, has been found to mineralize pyrene and benzo[*a*]pyrene. A phylogenetic analysis based upon 16S rRNA classified these two strains within the fast-growing group of mycobacteria [15]. An analysis of the degradation products of pyrene, benz[*a*]anthracene and benzo[*a*]pyrene produced by these mycobacteria enabled the degradation pathways to be proposed [16,17•]. Another *Mycobacterium* strain, BB1, is also able to grow on phenanthrene, pyrene and fluoranthene. Although mycobacteria are known for their comparatively slow growth, their growth on PAHs is faster than that of other microorganisms: the growthrate of *Mycobacterium* sp. BB1 on pyrene was 0.056 h^{-1}, whereas that of *Rhodococcus* sp. strain UW1 was 0.023 h^{-1} [18].

Sphingomonas paucimobililis strain EPA 505 isolated from a creosote-contaminated site was found to use fluoranthene as the sole source of carbon and energy [19]. The strain grown on fluoranthene can degrade a variety of PAHs, including pyrene, benz[*a*]anthracene, chrysene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, and dibenz[*a,h*]anthracene [20].

PAH-degrading fungi

A diverse group of fungi have the ability to nonspecifically degrade a wide range of PAHs, some of them degrading lignin (ligninolytic) while others are nonligninolytic. White rot fungi are ligninolytic, and are known to degrade PAHs that include the potent carcinogen benzo[*a*]pyrene and to detoxify PAH-polluted soils and sediments. A battery of extracellular enzymes responsible for the degradation of lignin, lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccases, is believed to be involved in the degradation of PAHs. Laccases are blue copper oxidases and catalyze the one-electron oxidation of organic substrates that is coupled to the four-electron reduction of molecular oxygen to water. Although laccases exhibit relaxed substrate specificity, they may be less important than LiPs and MnPs in the oxidation of PAHs [21].

The best-studied white rot fungus, *Phanerochaete chrysosporium*, produces multiple LiPs and MnPs. LiPs from *P. chrysosporium* catalyze the one-electron oxidation of PAHs to produce unstable aryl cation radicals. Different LiP isozymes exhibit different kinetic properties. These enzymes oxidize PAHs with ionization potential values

of less than 7.55 eV [22]. So far, 10 structural genes for LiP isozymes, designated *lipA* through *lipJ*, have been found [23]. The expression of 10 *lip* genes in anthracene-transforming soil cultures of *P. chrysosporium* has been examined by mRNA extraction from soil and the quantification of *lip* mRNA by competitive reverse-transcriptase-PCR (RT-PCR). The expression of the 10 *lip* genes was found to be regulated differently. The oxidation of anthracene proceeded constantly throughout the 25-day course of the experiment, suggesting that this compound was oxidized by several LiP isozymes [24].

MnPs are haem glycoproteins, and their synthesis is induced by Mn^{2+}. Mn^{2+} is also required for the catalytic reaction, because Mn^{3+} produced by the enzymatic oxidation of Mn^{2+} can oxidize lignin. The cDNAs (MnP1, MnP2a, MnP2b and MP-1) and genomic DNA (MnP1 and MnP2) of MnP isozymes in *P. chrysosporium* have been cloned and sequenced (see [25] for references). The crystal structure of one MnP has also been elucidated [26]. MnPs catalyze Mn^{2+}-dependent H_2O_2-independent lipid peroxidation. MnPs also catalyze the oxidation of PAHs in a reaction that requires Mn^{2+}, oxygen and unsaturated lipids. It is likely that the oxidation of high ionization potential PAHs such as phenanthrene is not mediated by Mn^{3+}, the direct product in MnP catalysis, but by lipid peroxidation-based co-oxidation [27]. In fact, in an *in vitro* system containing Mn^{2+}, unsaturated fatty acid and MnP, the oxidation of fluorene was inhibited by the free-radical scavenger butylated hydroxytoluene. Radicals generated from lipid hydroperoxide could react with PAHs according to several mechanisms, including hydrogen abstraction, electron abstraction and direct addition of the radical to an olefinic bond. It has been proposed that the initial oxidation of phenanthrene and fluorene proceeds by different mechanisms [28•]. The expression of three MnP isozyme genes in *P. chrysosporium* during a bench-scale PAH soil bioremediation experiment has been examined. mRNA was quantified by competitive RT-PCR with housekeeping mRNAs as controls. The expression of these three MnP genes was coordinately regulated under these growth conditions. *P. chrysosporium* cultured in soil caused the transformation of fluorene and the disappearance of chrysene in the early phase of fungal colonization, these activities being correlated with the degree of expression of the MnP genes [29•].

Another white rot fungus, *Pleurotus ostreatus*, probably mineralizes PAHs to CO_2 better than *P. chrysosporium*. *P. ostreatus* expresses laccase and manganese-inhibited peroxidase during fungal growth in the presence of various PAHs, but does not express LiP. The activities of these ligninolytic enzymes, however, did not correlate with those of PAH degradation enzymes [30]. *P. ostreatus* oxidized phenanthrene, the major intermediate formed being *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene. The absolute configuration of the predominant enantiomer produced is 9*R*,10*R*. One atom of $^{18}O_2$ was incorporated in *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene, indicating that a monooxygenase was involved in the formation of this intermediate. The inhibitor of P450 monooxygenases reduced the amount of *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene formed. From these observations, it has been suggested that a P450 monooxygenase is involved in the initial oxidation of phenanthrene [31•]. The presence of the P450 monooxygenase activity toward hydroxylate benzo[*a*]pyrene has also been demonstrated in *P. chrysosporium* [32•]. A non ligninolytic fungus, *Cunnninghamella elegans*, utilized cytochrome P450 monooxygenases for the initial attack on PAHs. The product, arene oxide, was further metabolized by epoxide hydrolase to form a dihydrodiol with the *trans*-configuration [1]. Thus, cytochrome P450 monooxygenases seem to play an important role in PAH biodegradation by fungi.

Adsorption of PAHs

In field tests on the bioremediation of contaminated soil with white rot fungi, it was found that PAHs with four or more fused rings were not degraded. This result was interpreted as indicating that these PAHs were adsorbed to the soil, and were not accessible to biodegradation [33]. Soil is a mixture of mineral and organic materials, and is an effective adsorbent for PAHs. The adsorption capacity is influenced by the nature of the soil, the soil moisture and other factors. Conceptually, the PAH-binding sites on soil could be classified into two groups: those on the mineral surface and those on organic matter. If the soil is wet, most of the mineral surface is occupied by water, and PAHs would be mainly adsorbed to the organic matter. In dry soil, however, adsorption of PAHs to both mineral surfaces and organic matter would occur. It has been demonstrated that a significant portion of adsorbed anthracene and other PAHs became nonextractable, especially when the water content of the soil was low. When the soil was dry, adsorbed PAHs were oligomerized and became nonavailable to biodegradation. For the polymerization of PAHs, the involvement of transient metals on the mineral surface has been suggested [34].

It is likely that some bacteria could utilize adsorbed PAHs as growth substrates. The availability to biodegradation of naphthalene adsorbed to soil has been examined by measuring the rate and extent of naphthalene degradation in soil-free and soil-containing systems. From the kinetics of the naphthalene utilization, it was concluded that *Pseudomonas putida* strain 17484 was able to directly access adsorbed naphthalene, while *Alcaligenes* sp. strain NP-Alk utilized only aqueous-phase naphthalene, with most of the soil-adsorbed fraction remaining unavailable [35,36•].

Effect of surfactants on the biodegradation of PAHs

Low water solubility and adsorption to soil are two major factors of PAHs that limit their availability to microor-

ganisms. Using surfactants that can render hydrophobic PAHs soluble may overcome these two problems for PAH bioremediation. A surfactant forms micelles at a concentration above the critical micelle concentration, and solubilized PAHs are entrapped inside the micelles. Conflicting results have, however, been reported concerning the effect of surfactants on biodegradation: surfactants stimulated biodegradation in some experiments whereas they were inhibitory in other experiments [37]. Several hypotheses have been proposed to explain the inhibitory effects of surfactants on biodegradation. One proposes that if microorganisms do not have direct access to PAHs inside the micelles, the mass transport of PAHs between these micelles and the aqueous phase would limit the biodegradation; however, from studies on the micellar-exchange dynamics of hydrophobic compounds with the aqueous phase, it was found that both the entrance and exit rates of PAHs were likely to be far higher than biodegradation rates [38]. Several studies have suggested that the use of surfactants below the critical micelle concentration would increase desorption and stimulate bioremediation. Other hypotheses have proposed either that the inhibition of biodegradation at higher surfactant concentrations would occur if the surfactant or solubilized PAH were toxic, or that the surfactant would be preferentially used by microorganisms as the source of carbon and energy.

To understand the effect of surfactants on bioremediation, a model of the effects of a surfactant on PAH dissolution and on biodegradation kinetics has been formulated by assuming that a bacterium has access only to aqueous-phase hydrocarbons and not to the hydrocarbons in micelles. The predictions from the model were tested by using phenanthrene-degrading *Pseudomonas stutzeri* P16. In an experiment, the addition of a surfactant, Tergitol NP-10, increased the growth rate of this organism. This result was in agreement with the prediction of the model using the measured kinetic and dissolution parameters [39•].

Using a mixed culture, the effect of nonionic surfactants on the biodegradation of phenanthrene has been examined, and the results compared with the values predicted by a mathematical model. It was concluded from such an analysis that phenanthrene in the micelles was bioavailable [40•]. In another system, four surfactants were tested to determine their effect on pyrene desorption and degradation by two *Pseudomonas* strains. Under unsaturated (but moist) conditions, one surfactant, Witconcol SN70, stimulated pyrene mineralization, whereas the same surfactant inhibited pyrene mineralization in soil slurries [41]. The reasons for the dual effect of the surfactant are not clear, but it is likely that the formulation of an appropriate model and the experimental determination of several parameters to test the model may be necessary to interpret this observation.

Conclusions

Although significant information concerning the biodegradation of PAHs has been accumulated, it is still too early to formulate a protocol for the rational design of a PAH bioremediation system. At present, targets for cleaning up PAH contamination are not necessarily clear. Because the operation of bioremediation costs a great deal, the complete removal of all contaminants up to the maximal level that current technology could achieve is not economically realistic. The goal of bioremediation should be clarified from the points of view of human health, protection of the global environmental and preservation of biodiversity. For example, the formation of nonextractable residues from organic pollutants during biodegradation and humification processes in soils is an important issue that needs to be addressed. Whether or not PAH contaminants that are incorporated into and are non extractable from humic substances should be removed by bioremediation is a debatable issue. To provide data for the basis of such discussions, the fate and toxicity of PAH carbon skeletons integrated in humic substances should be determined. Data concerning the bioconcentration of PAHs should also be accumulated [42].

Bioaugmentation is one option for bioremediation, especially when the number of appropriate microorganisms is not sufficient at a contaminated site [43]. Thus, improvement of the enumeration methods for PAH-degrading bacteria is important. As an alternative to the classical method [44], PCR-based technology for detecting specific PAH-degrading bacteria is under development [45]. Although the biochemical mechanisms for PAH degradation by microorganisms, and especially by fungi, have started to be elucidated in the past two years, it is not yet clear which organisms are most advantageous for detoxifying specific PAH compounds. Comparative studies should be conducted to rank different microorganisms in order of their ability.

As has been discussed, the effects of adding surfactants on PAH bioremediation are not yet predictable. Improved models will be necessary to more accurately predict the effects of surfactant addition on biodegradation.

Acknowledgements

The financial assistance of the New Energy and Industrial Technology Development Organization, Japan is gratefully acknowledged.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cerniglia CE: Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation* 1992, 3:351–368.

2. Fujikawa K, Fort FL, Samejima K, Sakamoto Y: Genotoxic potency in ***Drosophila melanogaster*** of selected aromatic amines and polycyclic aromatic hydrocarbons as assayed in the DNA repair test. *Mutat Res* 1993, 290:175–182.

3. Wilson SC, Jones KC: Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. *Environ Pollut* 1993, 81:229–249.

4. Coates JD, Anderson RT, Lovley DR: Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. *Appl Environ Microbiol* 1996, 62:1099–1101.

5. Coates JD, Anderson RT, Woodward JC, Phillips EJP, Lovley DR: Anaerobic hydrocarbon degradation in petroleum-contaminated harbor sediments under sulfate-reducing and artificially imposed iron-reducing conditions. *Environ Sci Tech* 1996, 30:2784–2789.

6. Harayama S, Kok M, Neidle EL: Functional and evolutionary relationships among diverse oxygenases. *Annu Rev Microbiol* 1992, 46:565–601.

7. Goyal AK, Zylstra GJ: Molecular cloning of novel genes for polycyclic aromatic hydrocarbon degradation from ***Comamonas testosteroni*** GZ39. *Appl Environ Microbiol* 1996, 62:230–236.

8. Iwabuchi T, Inomata-Yamauchi Y, Katsuta A, Harayama S: Isolation and characterization of marine ***Nocadioides*** capable of growing and degrading phenanthrene at 42˚C. *J Marine Biotech* 1997, in press.

9. Grifoll M, Selifonov SA, Gatlin CV, Chapman PJ: Actions of a versatile fluorene-degrading bacterial isolate on polycyclic aromatic compounds. *Appl Environ Microbiol* 1995, 61:3711–3723.

10. Trenz SP, Engesser KH, Fischer P, Knackmuss H-J: Degradation of fluorene by ***Brevibacterium*** sp. strain DPO 1361: a novel C-C bond cleavage mechanism via 1,10-dihydro-1,10-dihydroxyfluorene-9-one. *J Bacteriol* 1994, 176:789–795.

11. Kayser KJ, Bielaga-Jones BA, Jackowski K, Odusan O, Kilbane JJ II: Utilization of organosulphur compounds by axenic and mixed cultures of ***Rhodococcus rhodochrous*** IGTS8. *J Gen Microbiol* 1993, 139:3123–3129.

12. Demome SA, Oldfield C, Nash LJ, Young KD: Characterization of the desulfurization genes from ***Rhodococcus*** sp. strain IGTS8. *J Bacteriol* 1994, 176:1612–1614.

13. Piddington CS, Kovacevich BR, Rambosek J: Sequence and molecular characterization of a DNA region encoding the dibenzothiophene desulfurization operon of ***Rhodococcus*** sp. strain IGTS8. *Appl Environ Microbiol* 1995, 61:468–475.

14. Heitkamp MA, Cerniglia CE: Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. *Appl Environ Microbiol* 1988, 54:1612–1614.

15. Govindaswami M, Feldhake DJ, Kinkle BK, Mindell DP, Loper JC: Phylogenetic comparison of two polycyclic aromatic hydrocarbon-degrading mycobacteria. *Appl Environ Microbiol* 1995, 61:3221–3226.

16. Boldrin B, Tiehm A, Fritzsche C: Degradation of phenanthrene, fluorene, fluoranthene, and pyrene by a mycobacterium sp. *Appl Environ Microbiol* 1993, 59:1927–1930.

17. • Schneider J, Grosser R, Jayashimuhulu K, Xue W, Warshawsky D: Degradation of pyrene, benz[***a***]anthracene, and benzo[***a***]pyrene by ***Mycobacterium*** sp. strain RJGII-135, isolated from a former coal gasification site. *Appl Environ Microbiol* 1996, 62:13–19.

Mycobacterium sp. strain RJGII-135 degrades pyrene, benz[*a*]anthracene and benzo[*a*]pyrene. Degradation products of these PAHs were identified and the pathways for the degradation of these compounds were proposed.

18. Heitkamp MA, Freeman JP, Miller DW, Cerniglia CE: Pyrene degradation by a ***Mycobacterium*** sp.: identification of ring oxidation and ring fission products. *Appl Environ Microbiol* 1988, 54:2556–2565.

19. Mueller JG, Chapman PJ, Blattmann BO, Pritchard PH: Isolation and characterization of a fluoranthene-utilizing strain of ***Pseudomonas paucimobilis***. *Appl Environ Microbiol* 1990, 56:1079–1086.

20. Ye D, Siddigi MA, Maccubbin AE, Kumar S, Sikka HC: Degradation of polynuclear aromatic hydrocarbons by ***Sphingomonas paucimobilis***. *Environ Sci Tech* 1996, 30:136–142.

21. Thurston CF: The structure and function of fungal laccases. *Microbiology* 1994, 140:19–26.

22. Hammel KE, Kalyanaraman B, Kirk TK: Oxidation of polycyclic aromatic hydrocarbons and dibenzo[***p***]-dioxins by ***Phanerochaete chrysosporium*** ligninase. *J Biol Chem* 1986, 261:16948–16952.

23. Gaskell J, Stewart P, Kersten PJ, Covert SF, Reiser J, Cullen D: Establishment of genetic linkage by allele-specific polymerase chain reaction: application to the lignin peroxidase gene family of ***Phanerochaete chrysoporium***. *Bio-Technology* 1994, 12:1372–1375.

24. Bogan BW, Schoenike B, Lamar RT, Cullen D: Expression of ***lip*** genes during growth in soil and oxidation of anthracene by ***Phanerochaete chrysosporium***. *Appl Environ Microbiol* 1996, 62:3697–3703.

25. Matsubara M, Suzuki J, Deguchi T, Miura M, Kitaoka Y: Characterization of manganese peroxidases from the hyperlignolytic fungus IZU-154. *Appl Environ Microbiol* 1996, 62:4066–4072.

26. Sundaramoorthy M, Kishi K, Gold MH, Poulos TL: The crystal structure of manganese peroxidase from ***Phanerochaete chrysosporium*** at 2.06-Å resolution. *J Biol Chem* 1994, 269:32759–32767.

27. Moen MA, Hammel KE: Lipid peroxidation by the manganese peroxidase of ***Phanerochaete chrysosporium*** is the basis for phenthrene oxidation by the intact fungus. *Appl Environ Microbiol* 1994, 60:1956–1961.

28. • Bogan BW, Lamar RT, Hammel KE: Fluorene oxidation ***in vivo*** by ***Phanerochaete chrysosporium*** and ***in vitro*** during manganese peroxidase-dependent lipid peroxidation. *Appl Environ Microbiol* 1996, 62:1788–1792.

P. chrysosporium converts fluorene to 9-fluorenone. The same conversion was observed *in vitro* in a system containing Mn^{2+}, unsaturated fatty acid and *P. chrysosporium* MnP. The oxidation of fluorene *in vitro* was inhibited by a free-radical scavenger, burylated hydroxytoluene. These results indicated that a MnP-mediated lipid peroxidation system is responsible for the oxidation of fluorene.

29. • Bogan BW, Schoenike B, Lamar RT, Cullen D: Manganese peroxidase mRNA and enzyme activity levels during bioremediation of polycyclic aromatic hydrocarbon-contaminated soil with ***Phanerochaete chrysosporium***. *Appl Environ Microbiol* 1996, 62:2381–2386.

The expression of three MnP genes in *P. chrysosporium* growing on the mixture of PAH was examined using the competitive RT-PCR technique. The three MnP genes were coordinately expressed, and the level of expression of these genes was correlated with the speed of PAH degradation, suggesting that MnPs are responsible for the degradation of these PAHs.

30. Bezalel L, Hadar Y, Cerniglia CE: Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus ***Pleurotus ostreatus***. *Appl Environ Microbiol* 1996, 62:292–295.

31. • Bezalel L, Hadar Y, Fu PP, Freeman JP, Cerniglia CE: Metabolism of phenanthrene by the white rot fungus ***Pleurotus ostreatus***. *Appl Environ Microbiol* 1996, 62:2547–2553.

P. ostreatus oxidized phenanthrene to *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene. One atom of $^{18}O_2$ was incorporated in this product. The inhibitor of P450 monooxygenases reduced the formation of this intermediate. These results indicated that a P450 monooxygenase is involved in the initial oxidation of phenanthrene.

32. Masaphy S, Levanon D, Henis Y, Venkateswarlu K, Kelly SL:
• Evidence for cytochrome P-450-mediated benzo[**a**]pyrene hydroxylation in the white rot fungus ***Phanerochaete chrysosporium***. *FEMS Microbiol Lett* 1996, 135:51–55.
Evidence for the P450-mediated benzo[a]pyrene hydroxylase activity in *P. chrysosporium* is presented. Although P450 was suggested to have a role in lignin degradation in white rot fungi, no direct biochemical evidence for it has been reported. The presence of cytochrome P450 and P450-mediated benzo[a]pyrene hydroxylase activity in the white rot fungus *P. chrysosporium* was demonstrated.

33. Davis MW, Glaser JA, Evans JW, Lamar RT: Field evaluation of the lignin-degrading fungus ***Phanerochaete sordida*** to treat creosote-contaminated soil. *Environ Sci Tech* 1993, 27:2572–2576.

34. Karimi-Lofabad S, Pickard MA, Gray MR: Reactions of polynuclear aromatic hydrocarbons on soil. *Environ Sci Tech* 1996, 30:1145–1151.

35. Guerin WF, Boyd SA: Differential bioavailability of soil-sorbed naphthalene to two bacterial species. *Appl Environ Microbiol* 1992, 58:1142–1152.

36. Crocker FH, Guerin WF, Boyd SA: Bioavailability of naphthalene
• sorbed to cationic surfactant-modified smectite clay. *Environ Sci Tech* 1995, 29:2953–2958.
The availability for biodegradation of naphthalene adsorbed to soil was examined by measuring the rate and extent of naphthalene degradation in soil-free and soil-containing systems. From the kinetics of the naphthalene utilization, it was concluded that *P. putida* strain 17484 was able to directly access adsorbed naphthalene, whereas *Alcaligenes* sp. strain NP-Alk utilized only aqueous-phase naphthalene.

37. Rouse JD, Sabatini DA, Suflita JM, Harwell JH: Influence of surfactants on microbial degradation of organic compounds. *Crit Rev Environ Sci Technol* 1994, 24:325–370.

38. Chen S, Inskeep WP, Williams SA, Callins PR: Fluorescence lifetime measurements of fluoranthene, 1-naphthol, and napropamide in the presence of dissolved humic acid. *Environ Sci Tech* 1994, 28:1582–1588.

39. Grimberg SJ, Stringfellow WT, Aitken MD: Quantifying the
• biodegradation of phenanthrene by ***Pseudomonas stutzeri*** P16 in the presence of a nonionic surfactant. *Appl Environ Microbiol* 1996, 62:2387–2392.
Although micellized phenanthrene does not appear to be available directly to *P. stutzeri* P16, the addition of a surfactant increased the bacterial growth rate on phenanthrene. A model of the effect of the surfactant on the dissolution of phenanthrene was formulated by assuming that the bacterium has access only to aqueous-phase phenanthrene. The model predicted the enhancement of the bacterial growth by the addition of the surfactant.

40. Guha S, Jaffe PR: Biodegradation kinetics of phenanthrene
• partitioned into the micellar phase of nonionic surfactants. *Environ Sci Technol* 1996, 30:605–611.
Using a mixed culture, the effect of nonionic surfactants on the biodegradation of phenanthrene was examined, and the results were compared with the predicted values by a mathematical model. It was concluded that the phenanthrene in the micelles was bioavailable.

41. Thibault SL, Anderson M, Frankenberger WT Jr: Influence of surfactants on pyrene desorption and degradation in soils. *Appl Environ Microbiol* 1996, 62:283–287.

42. Hellou J, Mackay D, Fowler B: Bioconcentration of polycyclic aromatic compounds from sediments to muscle to finfish. *Environ Sci Tech* 1995, 29:2555–2560.

43. Vogel TM: Bioaugmentation as a soil bioremediation approach. *Curr Opin Biotechnol* 1996, 7:311–316.

44. Geiselbrecht AD, Herwig RP, Deming JW, Staley JT: Enumeration and phylogenetic analysis of polycyclic aromatic hydrocarbon-degrading marine bacteria from Puget Sound sediments. *Appl Environ Microbiol* 1996, 62:3344–3349.

45. Wang R-F, Luneau A, Cao W-W, Cerniglia CE: PCR detection of polycyclic aromatic hydrocarbon-degrading ***Mycobacteria***. *Environ Sci Tech* 1995, 30:307–311.