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Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation

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This article examines the importance of non-ligninolytic and ligninolytic fungi in the bioremediation of polycyclic aromatic hydrocarbon contaminated wastes. The research from the initial studies in Dave Gibson's laboratory to the present are discussed.

Keywords: fungi; biotransformation; polycyclic aromatic hydrocarbons; bioremediation

The defining moment that catapulted me into the field of microbial metabolism of polycyclic aromatic hydrocarbons (PAHs) was when I met David T Gibson, who was visiting Chase Van Baalen at The University of Texas Marine Science Institute (UTMSI), Port Aransas, Texas, for a seminar, a fishing trip, and a traditional poker game during the summer of 1976. Bob Tabita, a colleague of Dave's at the University of Texas at Austin, was also at UTMSI teaching marine microbial ecology, hitting home runs in the summer softball league, fishing and playing in the infamous poker games. My recollection is that Bob and Chase usually won most of Dave's and my chips in poker. This also continued to be a tradition over the years. Dave gave an outstanding lecture on the bacterial oxidation of aromatic hydrocarbons. His lecture, filled with enthusiasm and fun for science, was my first encounter with PAH chemistry. These ubiquitous pollutants, which comprise a class of chemicals consisting of three or more benzene rings fused in a linear, angular, or cluster arrangement (Figure 1), occur mostly as a result of the incomplete combustion of fossil fuels or from accidental discharge during the transport, use and disposal of petroleum products [65]. PAHs also occur as the result of the incineration of refuse and wastes and the burning of forests and agricultural residues [9]. Dave described how PAHs require metabolic activation to exert their mutagenic, carcinogenic and tumorigenic effects. He mentioned the research of James and Elizabeth Miller, who first showed that chemical carcinogens, after metabolic activation to electrophilic species, bind with nucleophilic groups of DNA, RNA and protein [81]. We now know that the metabolic activation of PAHs by cytochrome P450 monooxygenases is the first enzymatic step in the process leading to genotoxicity [48,49,57–59]. Enzymatic hydration of the non-K-region arene oxide via epoxide hydrolase then follows to form *trans*-dihydrodiols, which are further metabolized by cytochrome P450 monooxygenases to form dihydrodiol-epoxides, which alkylate DNA. For example, benzo[*a*]pyrene (BP) is metabolized in the C-7 and C-8 positions to *trans*-BP-7,8-dihydrodiol via cytochrome P450/epoxide hydrolase localized in the microsomal cell fraction. The proximate carcinogen is further metabolized to the potent carcinogen anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydroBP, which alkylates DNA (Figure 2). Another metabolic activation pathway involves a one-electron oxidation at the C-6 position of the BP ring system to form electrophilic cation radicals, which could also bind to cellular nucleophiles to form DNA adducts [18]. The generation of potentially toxic intermediates has become important to determine, since if bioremediation of PAH-contaminated sites is to become a viable technological option, we must consider whether the process is truly a detoxification pathway [97,106]. Because of their persistence in the environment and their genotoxicity, PAHs represent a health risk to humans; therefore, Dave discussed biodegradation principles and delighted the audience by telling the story of his quest to isolate and identify *cis*-dihydrodiols and the mechanism of enzymatic attack performed by bacteria. From his lecture, it was clear that Dave had a meticulous approach to scientific experimentation and high standards, which he has passed on to many of those who studied in his laboratory. After the seminar and over a couple of beers, Dave offered me a post-doctoral fellowship to conduct research in his laboratory at the University of Texas at Austin. I joined his group in the fall of 1976 and began what was the most critical period in developing my interest in microbial biochemistry.

When I arrived in the Microbiology Department at the University of Texas at Austin, my first research discussion with Dave was inspirational. He discussed the early observations of Auret *et al* [2], Ferris and co-workers [50,51], and Smith and Rosazza [98] that fungi metabolize aromatic compounds to metabolites similar to those formed by mammalian enzymes. They showed that the zygomycete, *Cunninghamella bainieri*, metabolized anisole, aniline and naphthalene by aryl hydroxylation and 4-nitroanisole by O-demethylation. For naphthalene biotransformation experiments, a naphthalene *trans*-dihydrodiol was formed, which suggested the possible presence of a cytochrome P450/epoxide hydrolase complex. Smith and Rosazza also provided evidence that several fungal species metabolized aromatic hydrocarbons in a way similar to mammals [98].

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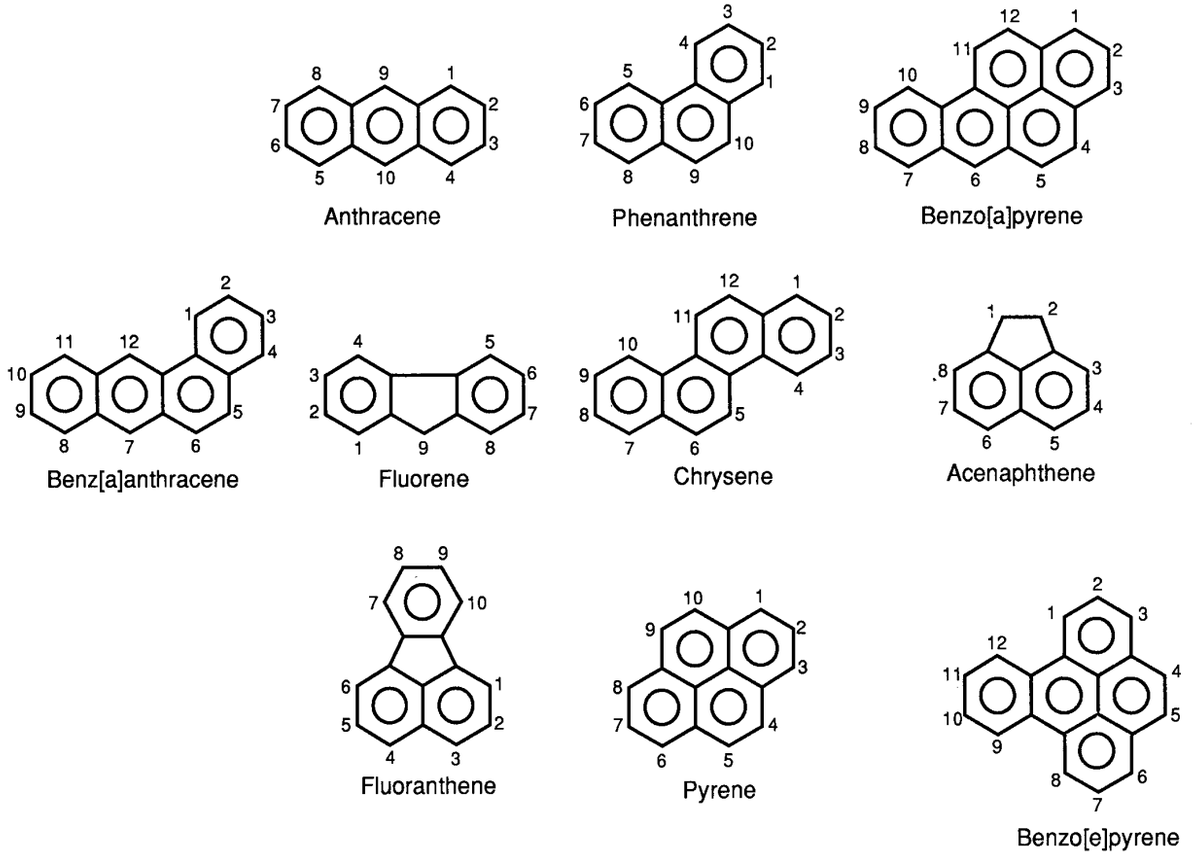

Figure 1 Chemical structures of polycyclic aromatic hydrocarbons.

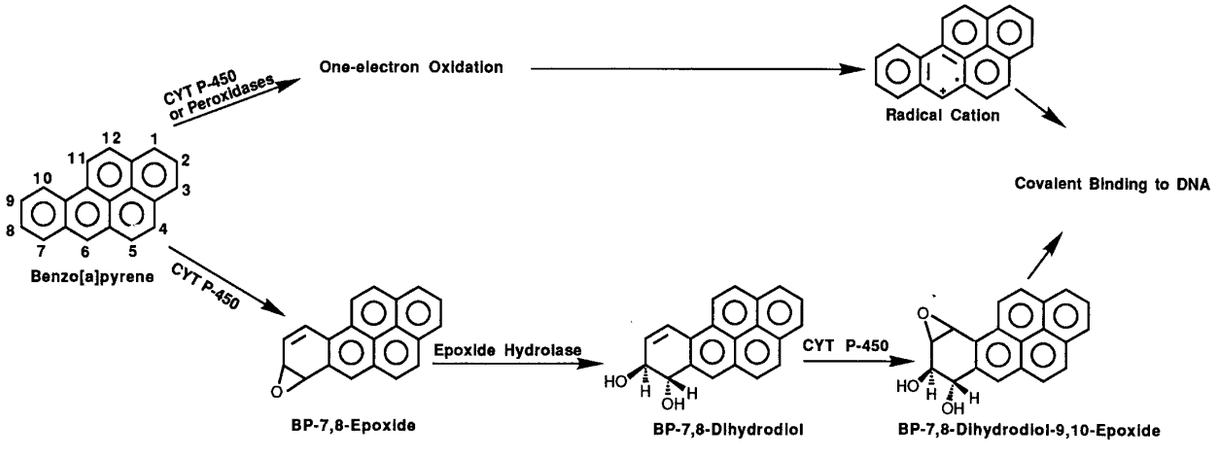

Figure 2 Metabolic activation pathways for the mammalian metabolism of benzo[*a*]pyrene.

They coined the phrase, 'microbial models of mammalian metabolism'. They suggested that microorganisms can be used to study drug metabolism with the added advantage of producing large quantities of metabolites, so that complete toxicological evaluation as well as rigorous structural elucidation studies can be obtained. Also, metabolites that are not easily synthesized by organic chemistry methods could easily be made by microorganisms.

Dave asked me to evaluate these papers and to outline experiments to test the hypothesis of the similarities and differences between the bacterial, fungal and mammalian metabolism of PAHs. In 1976, we knew that bacteria initially oxidize PAHs by incorporating both atoms of molecular oxygen into the PAH nucleus to form *cis*-dihydrodiols [54] (Figure 3). In contrast, it was known from the studies in Don Jerina's laboratory at NIH that mammals incorporate one atom of molecular oxygen into the PAH to form arene oxides that can either undergo enzymatic hydration by epoxide hydrolase to form *trans*-dihydrodiols or else rearrange non-enzymatically to form phenols [44].

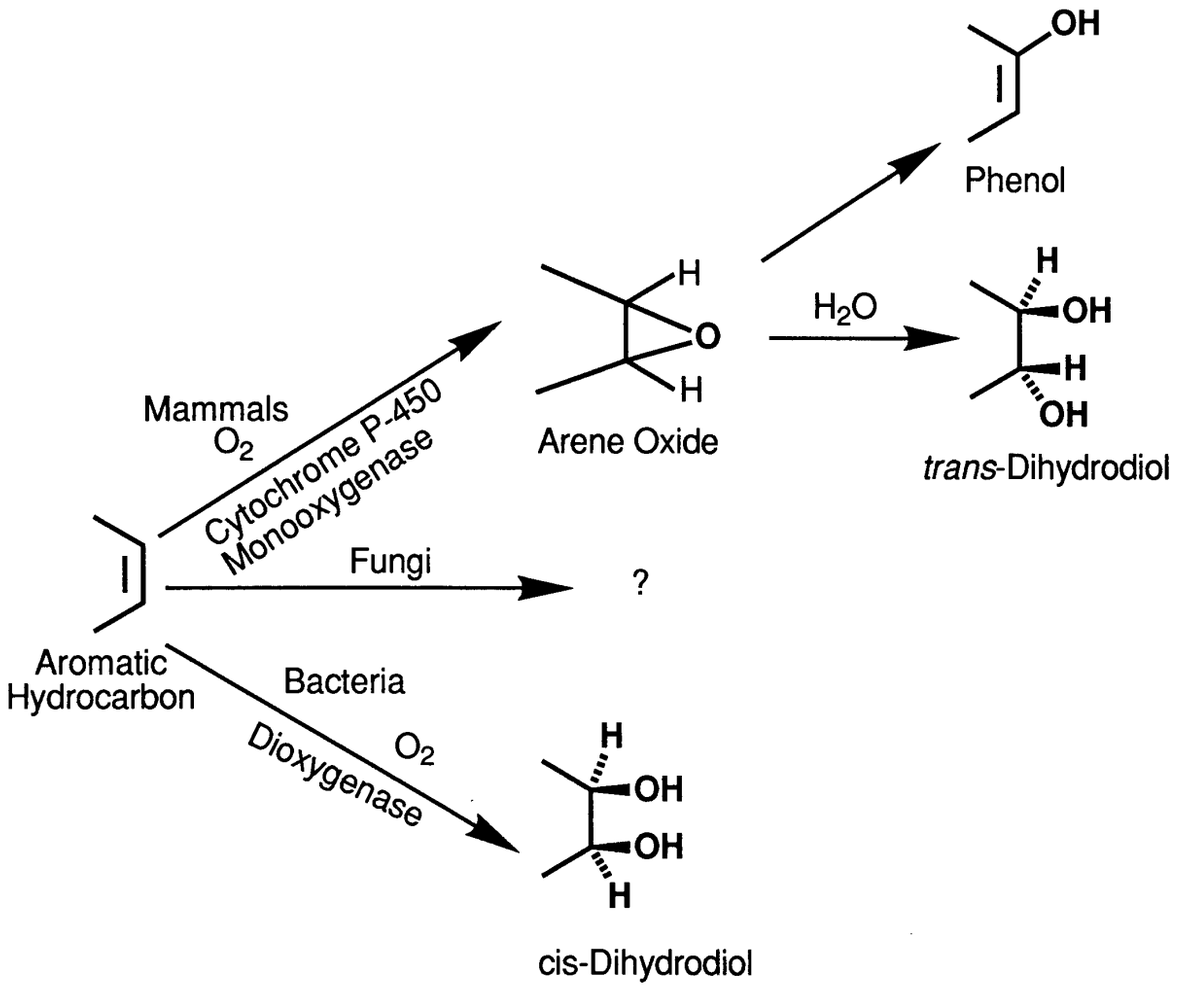

Figure 3 Different pathways used by bacteria and mammals in the metabolism of polycyclic aromatic hydrocarbons.

These observations led Dave Gibson to suggest that *cis* and *trans* hydroxylation of aromatic hydrocarbons represent basic differences in the metabolism of these substrates by procaryotic and eucaryotic organisms [55]. The question that I was asked to address was, 'Do fungi use the same or different reaction sequences as bacteria and mammals in metabolizing PAHs' (Figure 3)?

The atmosphere in Dave Gibson's laboratory was scientifically challenging, with all the technology available to develop and test the hypothesis. Certainly Dave directed a high-quality scientific research program, which attracted top students and post-doctoral fellows. Understanding the enzymatic mechanisms of the catabolism of aromatic hydrocarbons and the elucidation of chemical pathways were clear goals that I saw being pursued at this time.

Working with fungi was new to Dave's laboratory, which confirmed my desire to conduct research in this rewarding environment. The fungus that I used was *Cunninghamella elegans*, a zygomycete that was isolated from estuarine sediments obtained from the North Carolina coast [38]. This crude oil-degrading fungus was isolated in the laboratory by Dr Jerome J Perry, North Carolina State University. Dr Perry was my PhD mentor, a captivating man who instilled great enthusiasm for microbial physiology and ecology and delighted in telling his students stories about his days in Dr Jackson Foster's laboratory doing hydrocarbon oxidation studies. I was fortunate to begin my scientific career having Dr Perry as my mentor, since he laid the foundation necessary for an understanding of microbiology, which profoundly stimulated my research career. Since the initial isolation of *C. elegans* in 1972, this fungus has been used by many scientists to metabolize a wide variety of compounds that vary in properties from crude oil to tricyclic antidepressants [38,111].

Dave wanted me to do initial screening of metabolites formed from fungal biotransformation of PAHs that ranged in size from naphthalene to benzo[*a*]pyrene. Using classical biotransformation techniques and thin-layer chromatography (tlc) for detection of metabolites, we found that *C. elegans*, when grown on Sabouraud dextrose broth in the presence of naphthalene, biphenyl, anthracene, phenanthrene or benzo[*a*]pyrene, metabolized these PAHs to numerous metabolites. When I showed Dave the tlc plates, he said, 'Carl, that looks like a chemist's nightmare.' Dave's statement was profound, since that simple screening experiment has kept me busy for the last 20 years. The first detailed experiments on the fungal metabolism of PAHs was with naphthalene. Naphthalene was chosen as a model com-

pound, since its metabolism by mammals and bacteria had been examined extensively. We knew that the major route of metabolism of naphthalene by mammals involved the incorporation of one atom of molecular oxygen into the benzene nucleus to form naphthalene 1,2-oxide. The latter compound can undergo isomerization via the NIH shift to form 1-naphthol, enzymatic addition of water to form *trans*-1,2,dihydroxy-1,2-dihydronaphthalene (naphthalene *trans*-1,2-dihydrodiol), and the non-enzymatic addition of glutathione to form conjugated derivatives. In contrast, bacteria incorporate both atoms of molecular oxygen into naphthalene to form naphthalene 1,2-dihydrodiol with a *cis*-stereochemistry.

The initial biotransformation study in 1977 showed that *C. elegans* oxidized naphthalene to naphthalene *trans*-1,2-dihydrodiol, 1-naphthol, 2-naphthol, 4-hydroxy-1-tetralone and 1,2- and 1,4-naphthoquinones [30]. Subsequent studies showed that *C. elegans* also formed 1-naphthylglucuronide and 1-naphthyl sulfate [26]. A collaborative investigation in 1978 with Paul Szaniszlo's laboratory at the University of Texas at Austin showed that the ability of fungi to metabolize naphthalene was widespread among the five major fungal taxa [35]. The zygomycetes generally have the highest degradative ability amongst the fungi screened, which could be linked to cytochrome P450 involvement in ergosterol biosynthesis and fatty acid oxidation.

Further mechanism studies in 1983 in my laboratory at the National Center for Toxicological Research indicated that naphthalene 1,2-oxide was an intermediate of naphthalene oxidation catalyzed by cytochrome P450 monooxygenase via an NIH shift mechanism [22]. Experiments with $^{18}O_2$ indicated that the naphthalene *trans*-1,2-dihydrodiol that was formed contained one atom of molecular oxygen, which indicated a monooxygenase catalyzed reaction, while similar incubation with naphthalene and $H_2{}^{18}O$ indicated that the other oxygen atom in naphthalene *trans*-1,2-dihydrodiol was derived from water. Since these earlier studies, we have learned that *C. elegans* cannot utilize PAHs as sole sources of carbon and energy; however, it can cometabolize in a stereo- and regioselective manner a wide variety of PAHs, including acenaphthene [88], naphthalene [22,30], phenanthrene [23,41], anthracene [19,41], fluorene [89], fluoranthrene [87,90], chrysene [91], pyrene [36], benzo[*a*]pyrene [31–33,37], benzo[*e*]pyrene [86], benz[*a*]anthracene [25,34], and dibenzothiophene [96], as well as methylated [28,29,79,80] and nitro-substituted [27,82] analogs, to form *trans*-dihydrodiols, phenols, quinones, and dihydrodiol-epoxides (Figure 4). These metabolites are similar to phase I metabolites of xenobiotics formed by mammalian enzyme systems. However, a major difference between *C. elegans* and mammals is that the major enantiomer of the dihydrodiols formed by *C. elegans* has an *S,S* absolute configuration compared to the predominant *R,R* enantiomer formed by rat-liver microsomes [22,29,37,41,79,100]. The phase I enzymes, cytochrome P450 and epoxide hydrolase, have been detected in mycelial extracts of *C. elegans* [105,112]. However, the biochemical characterization, genetic regulation and endogenous functions have yet to be determined. We also found that the initial hydroxylation products could undergo phase II biotransformation to form glucuronides, sulfates and glucosides [17,23,26,31,36,86,87,91]. The isolation of these conjugated metabolites and the detection of aryl sulfotransferase, glutathione *S*-transferase, UDP-glucuronosyltransferase, and UDP-glucosyltransferase activities are indicative of a detoxification pathway, since conjugation products are considered to be less toxic than the parent PAH [40,112].The results suggest that *C. elegans* can be used to efficiently remediate PAH-contaminated soil.

A major breakthrough on the potential of fungi for use in PAH bioremediation was in 1985 when Bumpus *et al* [16] reported that the white-rot basidiomycete *Phanerochaete chrysosporium* partially degraded benzo[*a*]pyrene to carbon dioxide. The ability of *P. chrysosporium* to metabolize PAHs was attributed to the action of ligninolytic enzymes. This initial report has stimulated many other investigators to show that the lignin-degrading system of *P. chrysosporium*, which includes lignin peroxidase and manganese peroxidase, is important in PAH degradation [3,15]. Lignin peroxidases are extracellular enzymes that are induced during secondary metabolism under nutrient-deficient culture conditions. It has been shown that the lignin peroxidases oxidize a variety of PAHs with ionization potentials below 7.56 eV. Lignin peroxidases ionize aromatic compounds to create aryl cation radicals, which undergo further oxidation to form quinones [4,10–14,46,47,53,60–63,83,84]. For example, Hammel *et al* [61] showed that intact cultures of *P. chrysosporium* formed anthraquinone from anthracene. The anthraquinone was further metabolized to phthalic acid and carbon dioxide. Purified forms of lignin peroxidase and manganese peroxidase have also been shown to be capable of oxidizing anthracene, pyrene, fluorene and benzo[*a*]pyrene to the corresponding quinones [12,60–63,104] (Figure 5).

Recently other basidiomycetes, such as *Crinipellis stipitaria* [71,73–75], and white-rot fungi, such as *Trametes versicolor* [42,67], *Bjerkandera* sp [69] and *Pleurotus ostreatus* [5–8,72], have been shown to metabolize PAHs, such as phenanthrene, anthracene, pyrene, benzo[*a*]pyrene and fluorene. Interestingly, since in some cases these organisms produce little or no lignin peroxidase and manganese peroxidase, several enzymatic mechanisms could be used by white-rot fungi to degrade PAHs. For examples, Collins *et al* [43] have shown that purified laccase isozymes from *T. versicolor* oxidized anthracene and benzo[*a*]pyrene. Anthraquinone was identified as the major end product of anthracene oxidation. Similarly, PAHs with ionization potentials below 7.45 eV, such as anthracene and benzo[*a*]pyrene, could be oxidized by laccase, whereas fluorene and phenanthrene were not oxidized. Sutherland *et al* [102] also suggested another pathway in the metabolism of phenanthrene by *P. chrysosporium* through cytochrome P450 monooxygenase and epoxide hydrolase to form phenanthrene *trans*-9,10-dihydrodiol. Recently, a cytochrome P450 gene has been cloned and identified in *P. chrysosporium* [70].

A collaboration between my laboratory and that of Yitzhak Hadar at the Hebrew University of Jerusalem, Rehovot, has indicated that the edible basidiomycete, *Pleurotus ostreatus* (the oyster mushroom), can degrade a wide range of PAHs that include anthracene, phenanthrene, pyrene, fluorene, and benzo[*a*]pyrene [5–8]. *P. ostreatus*

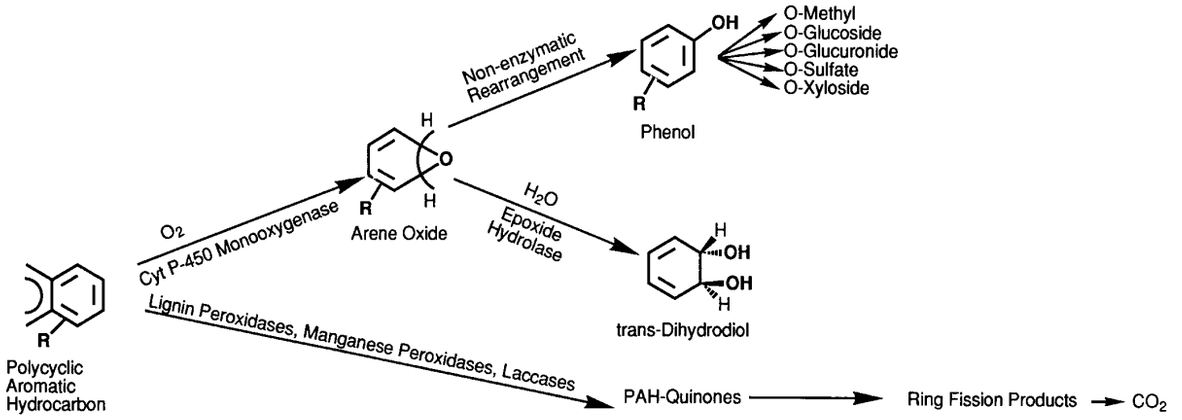

Figure 4 Pathways for the fungal metabolism of polycyclic aromatic hydrocarbons.

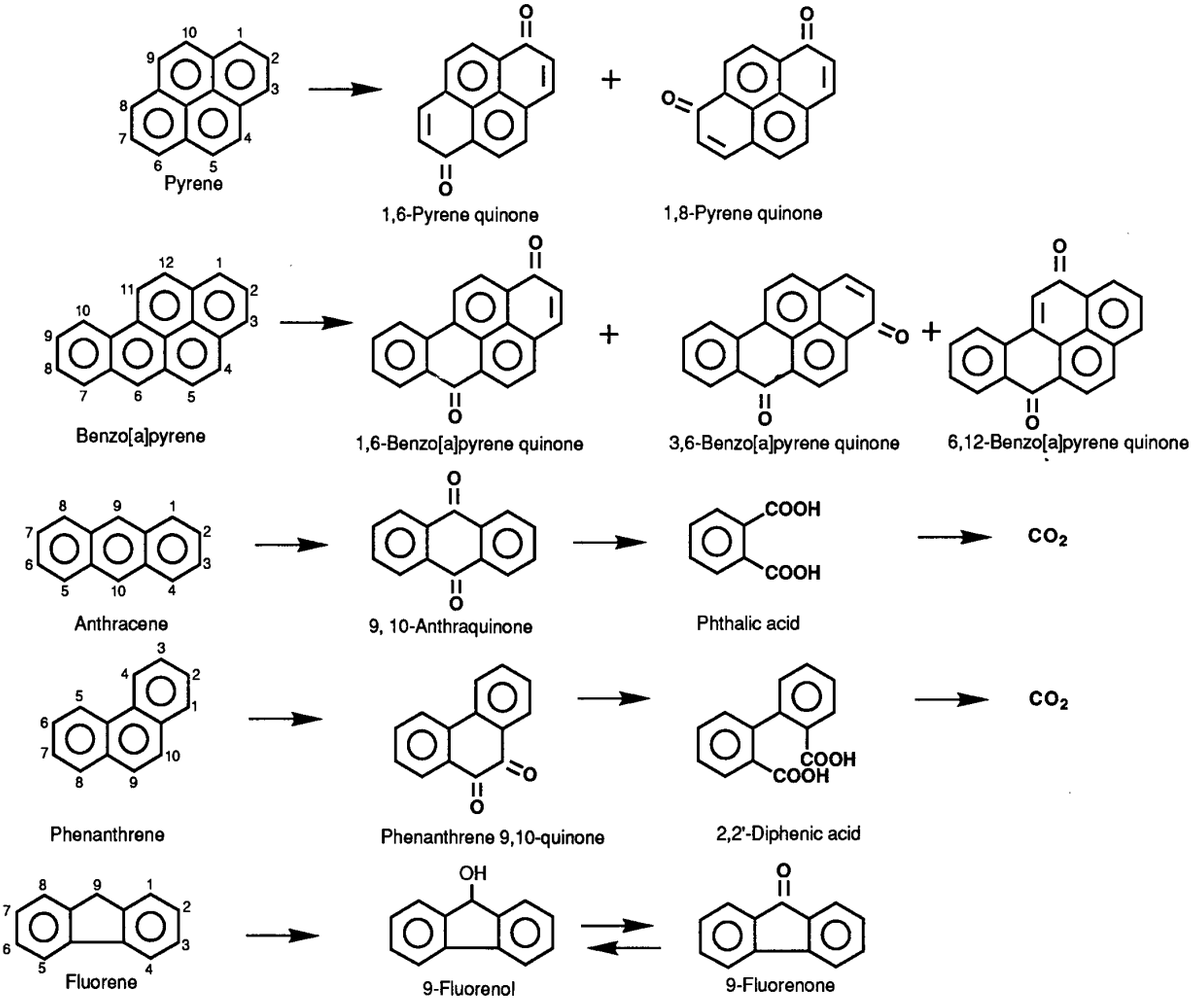

Figure 5 Oxidation of polycyclic aromatic hydrocarbons by ligninolytic fungi.

differs from *P. chrysosporium* in its lignin degradation mechanism, since it does not have lignin peroxidase activity. Based on the isolation of chemical intermediates, enzyme assays, inhibitor studies and $^{18}O_2$ incorporation experiments, we suggested that *P. ostreatus* has at least two basic mechanisms for the oxidation of PAHs. The predominant pathway in the initial oxidation of PAHs is a cytochrome P450/epoxide hydrolase catalyzed reaction leading to the formation of *trans*-dihydrodiols. These metabolic steps are similar to those found in non-ligninolytic fungi,

Table 1 Polycyclic aromatic hydrocarbons oxidized by different species of fungi

Compound	Organisms	Reference
Acenaphthene	*Cunninghamella elegans*	
Anthracene	*Bjerkandera* sp, *Cunninghamella elegans, Naematoloma frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Pleurotus sajor-caju, Ramaria* sp, *Rhizoctonia solani, Trametes versicolor*	[1,5,11,19,41,43,52,61,67,69,88, 93,95,103]
Phenanthrene	*Aspergillus niger, Cunninghamella elegans, Naematoloma frowardii, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Syncephalastrum racemosum, Trametes versicolor*	[5,11,15,17,23,41,42,47,62,83,84, 93–95,102,104]
Fluoranthene	*Cunninghamella elegans, Naematoloma frowardii, Laetiporus sulphureus, Penicillium* sp, *Pleurotus ostreatus*	[87,88,93,95]
Fluorene	*Cunninghamella elegans, Laetiporus sulphureus, Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor*	[8,12–14,42,53,89,93]
Pyrene	*Aspergillus niger, Agrocybe aegerita, Candida parapsilopsis, Crinipellis maxima, Crinipellis perniciosa, Crinipellis stipitaria, Crinipellis zonata, Cunninghamella elegans, Fusarium oxysporum, Kuehneromyces mutablis, Marasmiellus ramealis, Marasmius rotula, Mucor* sp, *Naematoloma frowardii, Penicillium janczewskii, Penicillium janthinellum, Phanerochaete chrysosporium, Pleurotus ostreatus, Syncephalastrum racemosum, Trichoderma harzianum*	[5,36,63,66,71–76,92,94,95,110]
Benz[*a*]anthracene	*Candida krusei, Cunninghamella elegans, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Rhodotorula minuta, Syncephalastrum racemosum, Trametes versicolor*	[1,11,25,34,78]
Benzo[*a*]pyrene	*Aspergillus ochraceus, Bjerkandera adusta, Bjerkandera* sp, *Candida maltosa, Candida maltosa, Candida tropicalis, Chrysosporium pannorum, Cunninghamella elegans, Mortierella verrucosa, Naematoloma frowardii, Neurospora crassa, Penicillium janczewskii, Penicillium janthinellum, Phanerochaete chrysosporium, Phanerochaete laevis, Pleurotus ostreatus, Ramaria* sp, *Saccharomyces cerevisiae, Syncephalastrum racemosum, Trametes versicolor, Trichoderma* sp, *Trichoderma viride*	[5,11,16,24,27,31–33,43,45,52,56,60,64,76,77, 95,107–109]
Chrysene	*Cunninghamella elegans, Penicillum janthinellum, Syncephalastrum racemosum*	[68,91]
Benzo[*e*]pyrene	*Cunninghamella elegans*	[86]

such as *C. elegans*. For example, *P. ostreatus* formed anthracene *trans*-1,2-dihydrodiol [8] predominantly in the *S,S* configuration, but pyrene *trans*-4,5-dihydrodiol [8] and phenanthrene *trans*-9,10-dihydrodiol [7] in the *R,R* configurations when metabolizing anthracene, pyrene and phenanthrene, respectively. These results indicated that *P. ostreatus* initially metabolized PAHs by reactions similar to those previously reported for *C. elegans*. However, in contrast to non-ligninolytic fungi, *P. ostreatus* has another oxidative pathway that can cleave the aromatic ring and mineralize the PAHs, as do ligninolytic fungi such as *P. chrysosporium*. The roles of laccases, manganese peroxidase and other ring cleavage enzymes in PAH degradation by *P. ostreatus* remain to be determined.

Since the initial studies in Dave Gibson's laboratory, a considerable amount of data have been reported by many laboratories on the importance of fungi in the metabolism of PAHs [20,21,39,85,99,101]. A summary of our current knowledge is listed below:

(1) A wide variety of fungi have been shown to metabolize PAHs that range in size from two to six rings (Tables 1 and 2). From the many PAH-degrading strains, the zygomycete *Cunninghamella elegans*, the ascomycetes *Aspergillus niger* and *Penicillium* sp, the white-rot basidiomycetes *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, and *Bjerkandera* sp and the brown rot basidiomycete *Lentinus lepideus* have exhibited significant potential to metabolize PAHs.
(2) Fungi do not utilize PAHs as the sole source of carbon and energy, and therefore the medium must be supplemented with an additional carbon source to allow fungi to metabolize them.
(3) The initial metabolism of PAHs by nonbasidiomycete fungi has been shown to result only in the oxidation of the PAHs; generally, PAHs are not mineralized by these strains.
(4) Some white-rot basidiomycetes have the ability to cleave the aromatic rings and mineralize PAHs.
(5) Generally, non-ligninolytic fungi metabolize PAHs to dihydrodiols, phenols, quinones and dihydrodiol epoxides. Conjugation products, like glucuronides, xylosides, glucosides and sulfates have also been reported. Conjugation pathways lead to detoxification, whereas the oxidation products like quinones or dihydrodiol epoxides may be bioactive and toxic.
(6) Several enzymatic systems are involved in the fungal metabolism of PAHs. They include intracellular cytochrome P450 and extracellular lignin peroxidase, manganese peroxidase and laccase.

Table 2 Metabolites produced from polycyclic aromatic hydrocarbons by fungi

Compound	Metabolites	References
Acenaphthene	1-Acenaphthenone, 1,2-Acenaphthenedione, *cis*-1,2-Dihydroxyacenaphthene, *trans*-1,2-Dihydroxyacenaphthene, 1,5-Dihydroxyacenaphthene, 6-Hydroxyacenaphthenone	[88]
Anthracene	Anthracene *trans*-1,2-Dihydrodiol 1-Anthrol, 9,10-Anthraquinone, Phthalate, Glucuronide, Sulfate and Xyloside conjugates of hydroxylated intermediates	[8,19,41,42,52,61,67,103]
Phenanthrene	Phenanthrene *trans*-1,2-dihydrodiol Phenanthrene *trans*-3,4-dihydrodiol Phenanthrene *trans*-9,10-dihydrodiol Glucoside conjugate of 1-phenanthrol 1-,2-,3-,4-, and 9-phenanthrol 1-methoxyphenanthrene, Phenanthrene-9,10-quinone 2,2′-Diphenic acid	[7,17,23,41,62,94,95,102,104]
Fluoranthene	Fluoranthene *trans*-2,3-dihydrodiol, 8 and 9-Hydroxyfluoranthene *trans*-2,3-dihydrodiols, Glucoside conjugates of hydroxylated intermediates	[87,90]
Chrysene	2-Chrysenyl sulfate 2-Hydroxy-8-chrysenylsulfate Chrysene *trans*-1,2-dihydrodiol	[68,91]
Benz[*a*]anthracene	Benz[*a*]anthracene *trans*-3,4-dihydrodiol, Benz[*a*]anthracene *trans*-8,9-dihydrodiol, Benz[*a*]anthracene *trans*-10,11-dihydrodiol, Phenolic and tetrahydroxy derivatives of benz[*a*]anthracene, Glucuronide and Sulfate conjugates of hydroxylated intermediates	[25,34]
Fluorene	9-Fluorenone 9-Fluorenol 2-Hydroxy-9-fluorenone	[8,12,89]
Pyrene	1,6-Pyrenequinone 1,8-Pyrenequinone Glucoside conjugates 1-Pyrenol 1,6-dihydroxypyrene 1,8-dihydroxypyrene 1-Pyrene sulfate 1-Hydroxy-8-pyrenyl sulfate 6-Hydroxy-1-pyrenyl sulfate Pyrene *trans*-4,5-Dihydrodiol	[8,36,63,75,76,94,110]
Benzo[*a*]pyrene	Benzo[*a*]pyrene *trans*-4,5-dihydrodiol Benzo[*a*]pyrene *trans*-7,8-dihydrodiol Benzo[*a*]pyrene *trans*-9,10-dihydrodiol Benzo[*a*]pyrene-1,6-quinone Benzo[*a*]pyrene-3,6-quinone Benzo[*a*]pyrene-6,12-quinone 3-Hydroxybenzo[*a*]pyrene 9-Hydroxybenzo[*a*]pyrene 7β,8α,9α,10β-tetrahydrobenzo[*a*]pyrene, 7β,8α,9α,10β-tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene, Benzo[*a*]pyrene 7,8-dihydrodiol-9,10-epoxide, Glucuronide and Sulfate conjugates of hydroxylated intermediates	[31–33,37,60,76,77,108]
Benzo[*e*]pyrene	3-Benzo[*e*]pyrenyl sulfate 10-Hydroxy-3-benzo[*e*]pyrenyl sulfate Benzo[*e*]pyrene-3-0-β-glucopyranoside	[86]

(7) Fungal metabolism of PAHs is highly regio- and stereoselective and in many cases is similar to mammalian metabolism.
(8) Fungi do not metabolize or mineralize PAHs as rapidly as bacteria.
(9) If the initial ring oxidation reaction is the rate-limiting step for the bacterial degradation of PAHs, production of hydroxylated intermediates by fungi will be important for the bioremediation of PAH-polluted sites, since it could accelerate the mineralization of these compounds.
(10) Besides mineralization of PAHs, fungi produce metabolites with higher water solubility and enhanced chemical reactivity, which could enhance the mineralization of these compounds by indigenous soil bacteria.
(11) Mineralization and formation of bound residues (humification) of PAHs by fungi might be considered a detoxification process, since they either completely degrade the PAH or have the potential to render them unavailable and therefore lessen the human toxicity.

From the early studies in Dave Gibson's laboratory to the present, we know that non-ligninolytic and ligninolytic fungi could be efficacious in the bioremediation of PAH-contaminated wastes. Efficient and cost-effective bioremediation should include either complete mineralization of the PAHs or at least biotransformation to less hazardous compounds. Generally fungal rates of degradation of PAHs are slow and inefficient compared to bacteria; however, since fungi have the ability to hydroxylate a wide variety of PAHs, their ecological role could be significant since these polar intermediates can be mineralized by soil bacteria or detoxified to innocuous compounds [66]. Furthermore, fungi have an advantage over bacteria since the fungal mycelium could grow into the soil and distribute itself through the solid matrix to degrade the PAHs. To optimize the biodegradative potential of fungi, extensive research on the enzymes involved in PAH degradation pathways and on the molecular genetics and biochemistry of catabolic pathways needs to be done.

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