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Detoxification of polycyclic aromatic hydrocarbons by fungi

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SUMMARY

The polycyclic aromatic hydrocarbons (PAHs) are a group of hazardous environmental pollutants, many of which are acutely toxic, mutagenic, or carcinogenic. A diverse group of fungi, including *Aspergillus ochraceus*, *Cunninghamella elegans*, *Phanerochaete chrysosporium*, *Saccharomyces cerevisiae*, and *Syncephalastrum racemosum*, have the ability to oxidize PAHs. The PAHs anthracene, benz[a]anthracene, benzo[a]pyrene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene, as well as several methyl-, nitro-, and fluoro-substituted PAHs, are metabolized by one or more of these fungi. Unsubstituted PAHs are oxidized initially to arene oxides, *trans*-dihydrodiols, phenols, quinones, and tetralones. Phenols and *trans*-dihydrodiols may be further metabolized, and thus detoxified, by conjugation with sulfate, glucuronic acid, glucose, or xylose. Although dihydrodiol epoxides and other mutagenic and carcinogenic compounds have been detected as minor fungal metabolites of a few PAHs, most transformations performed by fungi reduce the mutagenicity and thus detoxify the PAHs.

INTRODUCTION

Polycyclic aromatic hydrocarbons are a large group of xenobiotic pollutants that consist of benzene rings fused into various arrangements (Fig. 1). They are commonly released into air, soil, and water by coal mining, oil drilling, and the burning of wood and fossil fuels [9,10,29]. PAHs may contaminate fresh and dried foods exposed to sources of pollution [41,66,77]; they are also produced during cooking by methods such as charcoal broiling [41,62] and pan frying [78].

Several PAHs and their biotransformation products are toxic to living cells [9,41,67]. Although naphthalene binds to cells of the marine yeast *Candida lipolytica* and enhances their growth rate [37,38], it reduces the growth of many soil fungi [4,71]. Other PAHs are also taken up by fungi [66] but their effects on fungal growth are largely unknown. Many PAHs are mutagenic in bacterial and animal cells and carcinogenic for animals [41,81].

A number of comprehensive reviews have been written on the microbial metabolism of PAHs [9,10,29,48,49]. Recent reviews dealing specifically with metabolism by fungi include those describing the cytochromes P-450 of yeasts that oxidize benzo[a]pyrene [59], the degradation of xenobiotic pollutants by white-rot fungi [1,6,51], and the metabolism of aromatic hydrocarbons by yeasts and filamentous fungi [34]. Some of these fungi, such as the zygomycetous fungus *Cunninghamella elegans*, have been studied extensively to investigate possible pathways for the detoxification of environmental pollutants [9,54].

This article will outline the principal types of compounds that are usually formed during the metabolism of PAHs by fungi and will discuss what is known about the toxicity of these fungal metabolites.

METABOLITES PRODUCED BY FUNGI FROM PAHs

General comments

The PAH metabolites produced by fungi include phenols, *trans*-dihydrodiols, quinones, and tetralones (Fig. 2) [14,23,28]. Arene oxides have not been isolated from culture media but they appear to be intermediates in the formation of *trans*-dihydrodiols and phenols [11,29,34]. All of these fungal metabolites are produced by reactions similar to those known in pharmacology as phase-1 metabolism [40]. Phase-2 metabolism [40], in which these metabolites are conjugated with sulfate, glucuronic acid, or other moieties, also occurs in fungi [18]. The reactions of either of these phases are considered to be steps in detoxification if the products are less toxic than the original PAHs [35,65].

Most of the metabolites produced from PAHs by fungi are less toxic to other organisms than the parent compounds so that the net result is detoxification [35,65].

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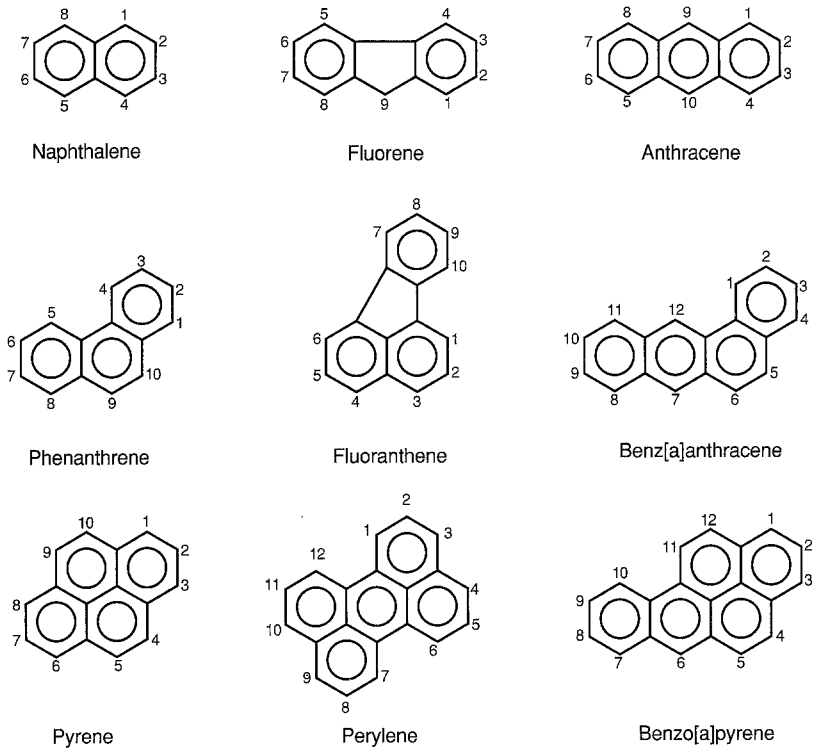

Fig. 1. Chemical structures of representative polycyclic aromatic hydrocarbons.

Small amounts of mutagenic and carcinogenic compounds, however, are formed during the fungal metabolism of a few unsubstituted and methyl-substituted PAHs [16,25,65].

Arene oxides

The first step in the fungal metabolism of an unsubstituted PAH is ring epoxidation by a monooxygenase enzyme complex [43,44]. The product of epoxidation is an unstable arene oxide, such as naphthalene 1,2-oxide (Fig. 2) [11]. Arene oxides are immediately either hydrated by epoxide hydrolase to *trans*-dihydrodiols or rearranged nonenzymatically to phenols [29,34]. The arene oxides have not been isolated directly from fungal culture media but naphthalene 1,2-oxide has been shown indirectly in *Cunninghamella elegans* by isotopic labelling

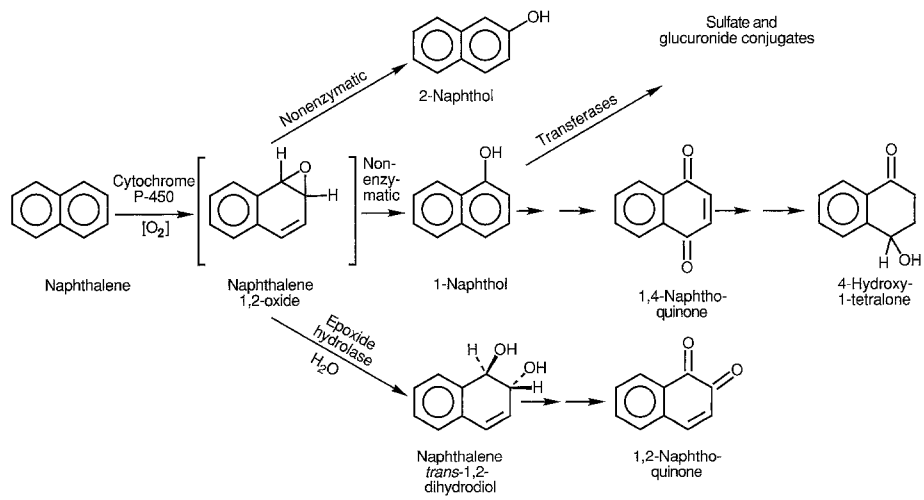

Fig. 2. Metabolism of naphthalene by fungi. Fig. adapted from [23].

experiments [11]. The toxicity of some arene oxides is less than that of the original PAHs; for example, synthetic benzo[a]pyrene 7,8-oxide is only about 20% as carcinogenic for mice as benzo[a]pyrene [81].

The monooxygenase enzyme complex that catalyzes the formation of arene oxides generally contains an inducible, membrane-bound enzyme, cytochrome P-450 [34,44,59]. The activity of this enzyme has been measured in several species, including *Cunninghamella bainieri* [43,44], *C. elegans* [24], *Aspergillus ochraceus* [42,47], and the yeast *Saccharomyces cerevisiae* [83,86,87]. Cytochrome P-450 probably occurs in most fungi.

Multiple isozymes of cytochrome P-450 have been found in *Aspergillus ochraceus* [47] and *Saccharomyces cerevisiae* [83,86]. One of the isozymes is induced in *S. cerevisiae* by benzo[a]pyrene [83,86,87]. Microsomal preparations of *S. cerevisiae* [83,85] and *A. ochraceus* [39,42,47] hydroxylate benzo[a]pyrene, presumably via unstable arene oxides. Low concentrations of one of the isozymes are found in cells grown without benzo[a]pyrene [56,57,86]. However, if *A. ochraceus* or *S. cerevisiae* is grown in a medium containing benzo[a]pyrene, the isozyme with activity on PAHs is induced in preference to other isozymes of cytochrome P-450 [47,58].

The purified cytochrome P-450 isozyme of *Saccharomyces cerevisiae* that is induced by benzo[a]pyrene [2,3] has been shown, by visible light spectrophotometry and tritium nuclear magnetic resonance (^{3}H-NMR) spectroscopy, to bind benzo[a]pyrene as a substrate [56,61,87]. The oxidation of benzo[a]pyrene has been demonstrated in vitro with a reconstituted monooxygenase complex containing purified NADPH, NADPH-cytochrome *c* reductase, cytochrome P-450 from *S. cerevisiae*, and a phospholipid [2,3,58].

Trans-Dihydrodiols

Epoxide hydrolase catalyzes the addition of a water molecule to an arene oxide to form a *trans*-dihydrodiol. This enzyme has been found in *Cunninghamella elegans* [82] and is presumed to occur in other fungi. One or more *trans*-dihydrodiols are produced by fungi from naphthalene (Fig. 2), anthracene, phenanthrene, fluoranthene, benz[a]anthracene, and benzo[a]pyrene (Table 1). The carcinogenicity of benzo[a]pyrene *trans*-9,10- and 4,5-dihydrodiols is much lower than that of the parent compound, but that of the *trans*-7,8-dihydrodiol is nearly as high [81].

Due to the complex fused ring structures of most PAHs, more than one *trans*-dihydrodiol isomer can be produced metabolically. Different fungi produce different isomers in laboratory cultures. For instance, phenanthrene is oxidized by *Cunninghamella elegans* mainly to the *trans*-1,2-dihydrodiol with small amounts of the *trans*-3,4- and 9,10-dihydrodiols [12,36], but it is oxidized initially by the white-rot fungus *Phanerochaete chrysosporium* to the *trans*-9,10- and 3,4-dihydrodiols [80].

TABLE 1

trans-Dihydrodiols produced from PAHs by fungi

Compound	References
Naphthalene *trans*-1,2-dihydrodiol	[14,23,24,43]
Anthracene *trans*-1,2-dihydrodiol	[8]
Phenanthrene *trans*-1,2-dihydrodiol	[36]
Phenanthrene *trans*-3,4-dihydrodiol	[36]
Phenanthrene *trans*-9,10-dihydrodiol	[12,80]
Fluoranthene *trans*-2,3-dihydrodiol	[72]
Benz[a]anthracene *trans*-3,4-dihydrodiol	[35]
Benz[a]anthracene *trans*-8,9-dihydrodiol	[15]
Benz[a]anthracene *trans*-10,11-dihydrodiol	[35]
Benzo[a]pyrene *trans*-4,5-dihydrodiol	[39]
Benzo[a]pyrene *trans*-7,8-dihydrodiol	[25,32,39]
Benzo[a]pyrene *trans*-9,10-dihydrodiol	[25,39]

Phenols

The nonenzymatic rearrangement of a PAH arene oxide in solution produces one or two isomeric phenols [29]. For instance, naphthalene 1,2-oxide produces predominantly 1-naphthol with a small amount of 2-naphthol (Fig. 2) [11,23,24]. Both 1- and 2-naphthol have been detected among the naphthalene metabolites of a wide variety of fungi [28,53,79]. The relative toxicity of naphthalene and 2-naphthol has been investigated for representative green algae, invertebrates, and fish. For 7 out of the 9 aquatic species tested, naphthalene is 1.5–6.4 times as toxic as 2-naphthol [67]. For cyanobacteria, however, both 1- and 2-naphthol are much more toxic than naphthalene [17].

Phenols are produced from naphthalene, anthracene, phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene by various fungi (Table 2). Unlike the parent compound

TABLE 2

Phenols produced from PAHs by fungi

Compound	References
1-Naphthol	[11,14,23,24,28,44,53]
2-Naphthol	[11,14,23,24,28,44]
3-Hydroxyphenanthrene	[80]
4-Hydroxyphenanthrene	[80]
9-Hydroxyphenanthrene	[80]
1-Hydroxypyrene	[30]
3-Hydroxybenzo[a]pyrene	[2,3,14,25,39,55,63]
9-Hydroxybenzo[a]pyrene	[14,25,39]

benzo[a]pyrene, 3- and 9-hydroxybenzo[a]pyrene demonstrate low mutagenicity in the *Salmonella typhimurium* reversion assay and do not appear to be carcinogenic [81].

Quinones

Several species of fungi produce 1,2- and 1,4-naphthoquinone from naphthalene (Fig. 2) [28]; other quinones are formed during the metabolism of pyrene and benzo[a]pyrene (Table 3). The probable metabolic pathway for the production of 1,2-naphthoquinone leads from naphthalene *trans*-1,2-dihydrodiol via 1,2-dihydroxynaphthalene; similarly, the probable metabolic pathway for 1,4-naphthoquinone leads from 1-naphthol via 1,4-dihydroxynaphthalene [23]. The respective diphenol intermediates, however, have not been detected in fungal culture media. Both 1,4- and 1,2-naphthoquinone are more toxic than naphthalene, at least for cyanobacteria [17].

Phanerochaete chrysosporium and *Cunninghamella elegans* oxidize pyrene to the 1,6- and 1,8-quinones [30,52]. These two species and *Aspergillus ochraceus* also oxidize the carcinogen benzo[a]pyrene to the 1,6-, 3,6-, and 6,12-quinones [25,39,50]. One of the enzymes responsible for quinone formation by *P. chrysosporium* has been identified; purified lignin peroxidase transforms benzo[a]pyrene to a mixture of all three quinones [50]. The benzo[a]pyrene quinones have only 3–4% as much tumor-initiating activity for mice as benzo[a]pyrene [81].

Tetralones

4-Hydroxy-1-tetralone, also designated as 4-hydroxy-3,4-dihydro(2*H*)naphthalenone (Fig. 2), is one of the metabolites produced from naphthalene by *Candida lipolytica*, *Cunninghamella* spp., *Neurospora crassa*, *Psilocybe* spp., *Syncephalastrum racemosum*, and several other fungi [14,23,28]. The intermediates in tetralone formation are not known but they may include 1-naphthol and 1,4-naphthoquinone [23,28].

TABLE 3

Quinones produced from PAHs by fungi

Compound	References
1,2-Naphthoquinone	[23,28]
1,4-Naphthoquinone	[23,28]
Pyrene-1,6-quinone	[30,52]
Pyrene-1,8-quinone	[30,52]
Benzo[a]pyrene-1,6-quinone	[25,39,50]
Benzo[a]pyrene-3,6-quinone	[25,39,50]
Benzo[a]pyrene-6,12-quinone	[50]

Dihydrodiol epoxides

When a monooxygenase catalyzes the further oxidation of a PAH *trans*-dihydrodiol, the result is a dihydrodiol epoxide (Fig. 3). Benzo[a]pyrene *trans*-7,8-dihydrodiol 9,10-oxide, one of the minor metabolites produced from benzo[a]pyrene by *Cunninghamella elegans* [26,48], is the ultimate carcinogenic and mutagenic metabolite of benzo[a]pyrene in mammals [81]. The same fungus also produces benzo[a]pyrene *trans*-9,10-dihydrodiol 7,8-oxide, which is less mutagenic [27].

Dihydrodiol epoxides can be metabolized further by

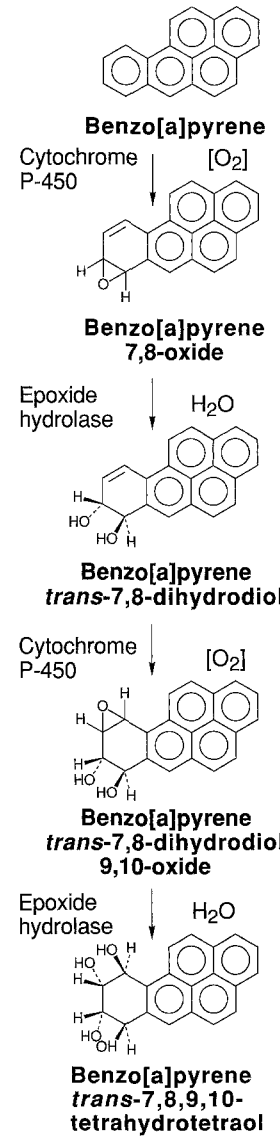

Fig. 3. Formation and hydrolysis of a carcinogenic dihydrodiol epoxide, benzo[a]pyrene *trans*-7,8-dihydrodiol 9,10-oxide, by *C. elegans*. Fig. adapted from [26].

epoxide hydrolase to tetrahydrotetraols, which are less mutagenic than the dihydrodiol epoxides [26,27].

Conjugates

During phase-2 metabolism [40], phenols and *trans* -dihydrodiols derived from PAHs are detoxified by conjugation with another molecule [18,65]. The conjugates produced by fungi, which are generally nontoxic to mammals [18] and nonmutagenic by the *Salmonella typhimurium* reversion assay [35], include sulfates, glucosides, glucuronides, and xylosides (Fig. 4). The toxicity of these conjugates to fungi has not been investigated but probably is low.

Sulfate conjugation, which is a common mammalian detoxification reaction for PAHs [81], is also performed by fungi [18]. *Cunninghamella elegans* produces 1-naphthyl sulfate (Fig. 4) and 1-anthryl sulfate from naphthalene and anthracene, respectively [8,18]. It also produces sulfate conjugates from benz[a]anthracene and benzo[a]pyrene [25,35].

Glucuronic acid conjugates of PAHs are detoxification products of both mammals and fungi [18,81]. A UDP-glucuronyltransferase in *Cunninghamella elegans*, which unlike the corresponding membrane-bound enzyme of mammals is soluble, catalyzes the conjugation of 3-hydroxybenzo[a]pyrene with glucuronic acid [25,82]. The same fungus also produces glucuronides from naphthalene (Fig. 4) and benz[a]anthracene [15,18,35].

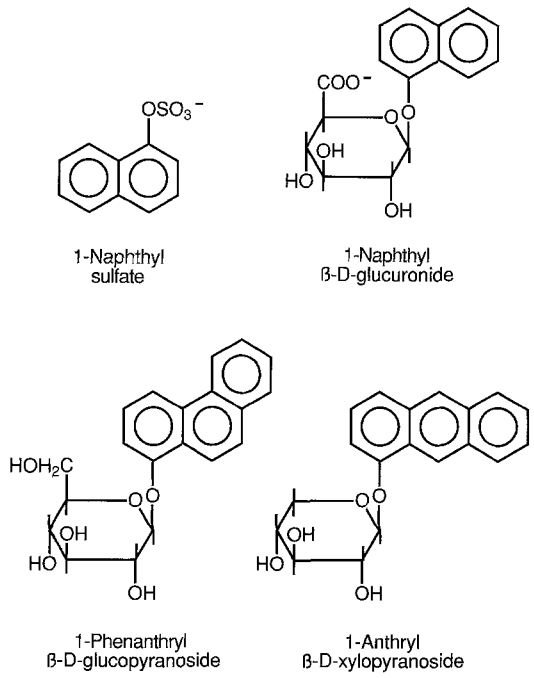

Fig. 4. Conjugates of polycyclic aromatic hydrocarbons with sulfate, glucuronic acid, glucose, and xylose.

Glucose conjugates are produced from PAHs by at least two fungi. *Cunninghamella elegans* and *Phanerochaete chrysosporium* form glucosides from phenanthrene (Fig. 4) [12,80], fluoranthene [72], and pyrene [30].

Xylose conjugates have recently been found among the metabolites of *Rhizoctonia solani* grown with anthracene (unpublished results). Results from our laboratory have tentatively identified two different isomeric xyloside conjugates of anthracene *trans*-1,2-dihydrodiol and a xyloside conjugate of 1-hydroxyanthracene (Fig. 4).

METABOLISM OF PAHs BY LIGNIN-DEGRADING FUNGI

White-rot fungi, which degrade lignin and cellulose in wood, produce nonspecific extracellular enzymes that can oxidize xenobiotics during growth on carbohydrates such as glucose or cellulose [1,6,51]. *Phanerochaete chrysosporium* and *Trametes versicolor* metabolize ^{14}C-labelled phenanthrene to $^{14}CO_2$ and several unidentified compounds [5,69]. Cultures of *P. chrysosporium* also produce $^{14}CO_2$ and unidentified metabolites from ^{14}C-labelled fluorene [5,46], pyrene [5,52], and benzo[a]pyrene [7,50,76].

Lignin peroxidase appears to be responsible for the initial steps in the oxidation by white-rot fungi of some, but not all, of these PAHs [5–7]. The purified lignin peroxidase isozyme H8 from *Phanerochaete chrysosporium* catalyzes the formation of quinones from anthracene, benz[a]anthracene, pyrene, perylene, and benzo[a]pyrene [50,52,76]. Since phenanthrene is oxidized by cultures of *P. chrysosporium* [5,69,80] but not by purified lignin peroxidase H8 [52], other enzymes, such as monooxygenases, must also be involved in the metabolism of some PAHs by white-rot fungi [80].

METABOLITES PRODUCED BY FUNGI FROM METHYLATED PAHs

Of the large number of methyl-substituted PAHs, only two will be considered here. These are 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene (Fig. 5), which have extremely high mutagenicity and carcinogenicity [16,65].

3-Methylcholanthrene is transformed by *Cunninghamella elegans* to secondary alcohols and ketones. These include 1- and 2-hydroxy-3-methylcholanthrene and 1- and 2-keto-3-methylcholanthrene [16]. Trace amounts of a carcinogenic metabolite, 1-hydroxy-3-methylcholanthrene *trans*-9,10-dihydrodiol, are also produced [16].

7,12-Dimethylbenz[a]anthracene is metabolized by several fungi. *Penicillium notatum*, *Syncephalastrum racemosum*, and *Cunninghamella elegans* hydroxylate 7,12-

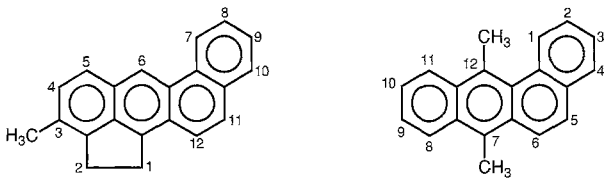

Fig. 5. Chemical structures of two methyl-substituted PAHs.

dimethylbenz[a]anthracene at either or both of the methyl groups [64,65,88]. However, *C. elegans* also oxidizes it to 7,12-dimethylbenz[a]anthracene *trans*-3,4-dihydrodiol, which is highly carcinogenic [64,65,84]. *Penicillium chrysogenum* and a *Mucor* sp. transform 7,12-dimethylbenz[a]anthracene to phenols [65]. Unlike the other four fungi tested, *S. racemosum* detoxifies 7,12-dimethylbenz[a]anthracene by conjugation with glucuronic acid and sulfate [65].

Fungi also metabolize several other methylated [13,20–22,31,45], nitrated [19,68,75], and fluorinated [33] PAHs. In general, the reactions are similar to those already discussed.

MUTAGENICITY OF FUNGAL METABOLITES OF PAHs

Some of the minor metabolites produced by fungi from unsubstituted and methylated PAHs are more mutagenic than either the parent compounds or the principal metabolites. These metabolites include benz[a]anthracene *trans*-3,4-dihydrodiol [15], benzo[a]pyrene *trans*-7,8-dihydrodiol 9,10-oxide [26], 7,12-dimethylbenz[a]anthracene *trans*-3,4-dihydrodiol [65,84], and 1-hydroxy-3-methylcholanthrene *trans*-9,10-dihydrodiol [16]. Despite the increased mutagenicity of these minor metabolites, the bulk of the fungal transformation products show reduced mutagenicity [35].

The mutagenicity of culture media containing PAHs decreases gradually during fungal growth. When *Cunninghamella elegans* is grown in Sabouraud's medium for 3 days in the presence of mutagens such as fluoranthene, benz[a]anthracene, 7,12-dimethylbenz[a]anthracene, 3-methylcholanthrene, or benzo[a]pyrene, the rat-liver –S9-mediated mutagenicity of the culture media in the *Salmonella typhimurium* reversion assay is greatly reduced but not totally eliminated [35,73]. In cultures of *Syncephalastrum racemosum* with 7,12-dimethylbenz[a]anthracene, the mutagenicity decreases gradually as the compound is converted to glucuronide and sulfate conjugates [65].

Although the sulfate, glucuronide, and glucoside conjugated produced from PAHs by fungi are nonmutagenic [35,73], other microorganisms in the environment have hydrolytic enzymes, such as sulfatases, glucuronidases, and glucosidases, that may conceivably remove these conjugative groups and restore toxicity. For example, certain bacteria have been shown to hydrolyze benzo[a]pyrene conjugates [70,74]. Purified β-glucuronidase also hydrolyzes the glucuronide conjugate of 3-hydroxybenzo[a]pyrene and increases binding to DNA [60]. For this reason, fungi and bacteria that cleave the aromatic rings of PAHs [5,29,69] should prove more useful for the bioremediation of toxic wastes containing PAHs than those that form conjugates.

CONCLUSIONS

Many species of fungi transform PAHs to *trans*-dihydrodiols, phenols, quinones, tetralones, conjugates, and other metabolites. Although small amounts of mutagenic and carcinogenic metabolites are formed during the metabolism of some PAHs, most fungal transformation products are less mutagenic than the original compounds. The utilization of selected microorganisms for the bioremediation of sites contaminated with PAHs should be a fruitful area for future investigation.

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