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Degradation of Organic Sulfur Compounds by a Coal-Solubilizing Fungus

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ABSTRACT

Paecilomyces sp. TLi, a coal-solubilizing fungus, was shown to degrade organic sulfur-containing coal substructure compounds. Dibenzothiophene was degraded via a sulfur-oxidizing pathway to 2,2'-dihydroxybiphenyl. No further metabolism of that compound was observed. Ethyl phenyl sulfide and diphenyl sulfide were degraded to the corresponding sulfones. A variety of products were formed from dibenzyl sulfide, presumably via free radical intermediates. Diphenyl disulfide and dibenzyl disulfide were cleaved to the corresponding thiols and other single-ring products. It was concluded that degradation of organic sulfur compounds by *Paecilomyces* involves an oxidative attack localized at the sulfur atom.

Index Entries: Coal; coal solubilization; dibenzothiophene; *Paecilomyces;* coal desulfurization.

INTRODUCTION

Certain microorganisms, including both bacteria and fungi, have been demonstrated to transform low-rank coals to soluble products (*1*). The study of fungal coal solubilization has been focused primarily on the fate of the carbon ring structure of low-rank coal. Little attention has been paid to the fate of heteroatoms such as sulfur and nitrogen, which are known to be covalently incorporated into the coal polymer, during coal biosolubilization by these organisms.

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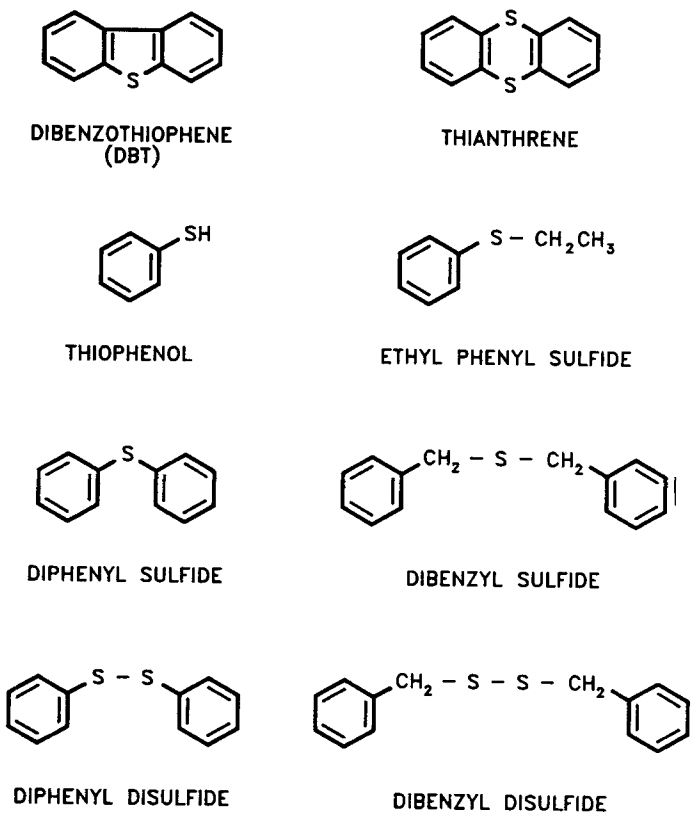

Fig. 1. Proposed pathways for microbial metabolism of DBT.

Organic sulfur within coal is known to exist in a variety of forms, including thiophenes, sulfides, disulfides, and thiols (*2*). Various microorganisms (primarily bacteria) have been examined for their potential utility in organic sulfur removal from high-rank coals (*2–4*). (Since low-rank coals have a very low fuel value, efforts have been directed toward the treatment of high-rank—generally bituminous—coals exclusively.) Preliminary screens for biodesulfurization activity have focused on degradation of dibenzothiophene (DBT), a thiophenic coal substructure compound. DBT may be degraded via a localized, oxidative attack on the sulfur atom, with release of free sulfate, or via an undesirable ring-destructive pathway that does not achieve sulfur removal (Fig. 1). Both sulfur-oxidizing (*3–5*) and ring-destructive (*6–9*) organisms have been described. These pathways are not, however, mutually exclusive: both have been shown to occur in a *Pseudomonas* sp. (*10*). There is, however, little information on the fate of other forms of organic sulfur in cultures of organisms active against DBT. Thianthrene (Fig. 2), a heterocyclic sulfide, was shown to be attacked by a purified fungal enzyme from *Phanerochaete chrysosporium* (*11*). Activity against thianthrene in vitro was localized at one sulfur atom with formation of the corresponding monooxide. The fate of thianthrene in cultures

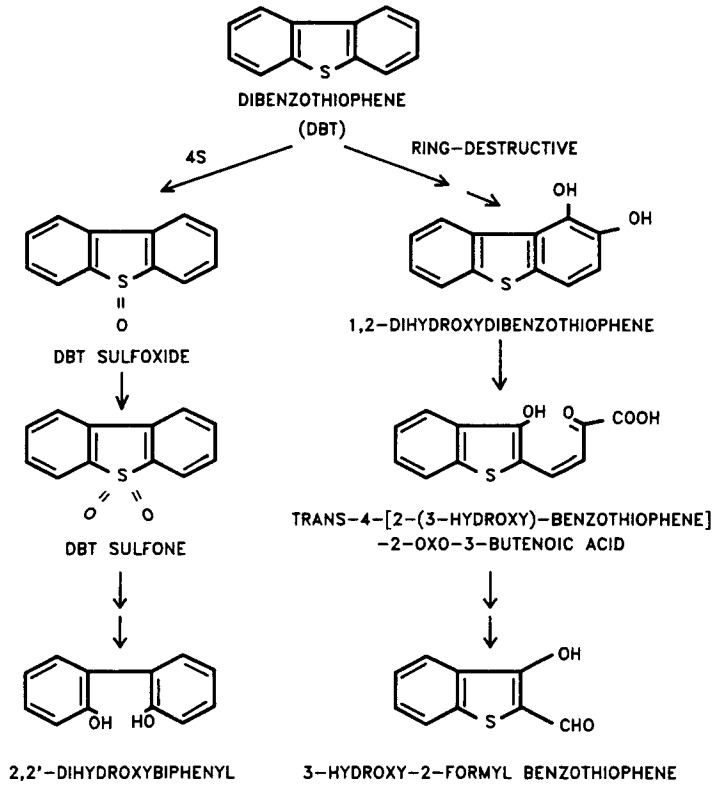

Fig. 2. Organic sulfur-containing coal model compounds.

was not determined. Intriguingly, *P. chrysosporium* was shown elsewhere to solubilize low-rank coal (*12*). The desulfurizing potential of other coal-solubilizing fungi has not been determined.

The experiments described here assessed the ability of a coal-solubilizing fungus to effect organic sulfur removal in vivo from compounds representative of coal substructures. The organism used in this work was *Paecilomyces* sp. TLi. The biosolubilizing activity of this organism has been well documented (*13–15*). Organic sulfur-containing test compounds used in this work included DBT, aryl sulfides, aryl disulfides, and thiophenol (Fig. 2). These compounds apparently undergo an oxidative attack at the sulfur atom in the absence of ring cleavage to form relatively stable products.

METHODS

Organism and Culture Conditions

Paecilomyces sp. TLi, isolated from a Texas lignite coal in this laboratory (*13*), was used in this work. Stock cultures were maintained on potato

dextrose agar slants. Experimental cultures (50-mL vol) were grown in submerged culture from conidial inocula as described previously (*14*). Initial spore density was 10^6/mL.

Spore inocula and growth media for experimental cultures were prepared in distilled deionized H_2O as delivered by a Milli-Q Water Purification System (Millipore Laboratories, Bedford, MA). All experimental cultures were grown in defined inorganic Czapek's medium (*14*) containing either glucose or maltose. This medium typically contains 2 m*M* sulfate (as Na_2SO_4). In the present work, sulfate was deliberately omitted from the medium; sulfate-containing medium components were replaced with their nitrate salts. Nonetheless, at least 9 μM sulfur (measured as sulfate) was present as a medium contaminant.

Cultures used in inorganic sulfur compound nutritional experiments were grown in medium containing 5% glucose and either sulfite, thiosulfate, elemental sulfur, or sulfide (sodium salts). The final concentration of sulfur was 2 m*M* in all cultures. Cultures were incubated at 30°C with agitation for 7 d. Duplicate cultures were harvested by centrifugation at 12,000*g* for 10 min at ambient temperature. Pellets were dried overnight at 95°C in a vacuum oven, and dry weights were determined.

Cultures used in organic sulfur compound nutritional experiments were grown in medium containing 5% maltose and 0.01% Tween-80, plus either DBT or thianthrene. The test compounds were dissolved in dimethylformamide (DMF), filter-sterilized, and added to the culture medium at the time of inoculation. The compounds formed a stable dispersion within the aqueous medium because of the presence of Tween-80. The test compounds were added to provide a final concentration of 2 m*M* sulfur in cultures. The final DMF concentration in cultures was 5%. DMF and Tween-80 were included in control cultures to which no test compound (or other sulfur source) was added. All cultures were incubated at 30°C with agitation for 7 d. Duplicate cultures were harvested by centrifugation at 12,000*g* for 10 min at ambient temperature. Pellets were dried overnight at 95°C in a vacuum oven and dry weights were determined.

Cultures used in organic sulfur compound degradation experiments were grown in medium containing 5% maltose and 0.01% Tween-80 as described above. Test compounds for this work were DBT, DBT sulfone, dibenzyl disulfide, dibenzyl sulfide, diphenyl disulfide, diphenyl sulfide, ethyl phenyl sulfide, thianthrene, and thiophenol. The nonsulfur-containing compound 2,2'-dihydroxybiphenyl was also included in this work. The organic compounds were dissolved in DMF (5% final concentration), filter-sterilized, and added to the culture medium to achieve a final concentration of 2 m*M* sulfur as described above. DMF and Tween-80 were included in control cultures to which no test compound was added. Uninoculated controls contained the test compound without spores. Cultures (controls and experimentals) were incubated at 30°C with agitation for up

to 30 d. At the end of the incubation period, entire cultures (pooled duplicates) were extracted with an equal volume of methylene chloride. The aqueous fraction was acidified to pH 2 and reextracted with an equal volume of 2-butanol. The methylene chloride extraction was designed to isolate fairly nonpolar aromatic compounds. More polar compounds (including the bulk of normal cell metabolites) were extracted into 2-butanol. The acidification step prior to 2-butanol extraction was designed to protonate acidic products, thus enhancing their solubility in the organic solvent. The methylene chloride and 2-butanol extracts were analyzed separately by gas and/or thin-layer chromatography.

Gas Chromatography (GC)

Capillary GC was carried out on a Hewlett-Packard Model 5890 instrument equipped with a fused silica capillary column with a bonded DB-Wax liquid phase (J & W Scientific Co., Folsom, CA) and split injector. Column dimensions were 60 m × 0.32 mm id (0.5-μm film thickness). The carrier gas was He, maintained at a column head pressure of 38 psi. Injector temperature was 240°C. The column temperature program was as follows: initial temperature, 100°C (5-min hold); increase at 5°C/min to a final temperature of 250°C (30-min hold). Products were detected by H_2-flame ionization at 320°C (20 psi). Chromatograms of experimental samples (culture extracts) were compared to those of authentic commercial standards. In some cases, product identification was confirmed by mass spectroscopy (MS) as described below.

GC/MS analyses were performed on a Hewlett-Packard 5985A instrument equipped with a fused silica capillary column with a bonded DB-5 liquid phase (J & W Scientific Co.) and splitless injector. Column dimensions were 30 m × 0.32 mm id (1.0-μm film thickness). The carrier gas was He, maintained at a column head pressure of 16 psi. The GC was interfaced directly to the ion source; the splitless injector and GC/MS transfer line were maintained at 280°C. The column temperature program was as follows: initial temperature, 100°C (5-min hold); increase at 5°C/min to a final temperature of 280°C (30-min hold). MS analysis was performed at 70 eV and a source temperature of 200°C.

Thin-Layer Chromatography (TLC)

TLC was carried out on Type 13181 silica gel plates (Eastman Kodak Co., Rochester, NY) containing a fluorescent indicator. Developing solvents were 1-heptane:acetone, 4:1 and 1:1. Phenolic products, which gradually became colored as a result of air oxidation, were easily detected under visible light. Other products were detected by viewing under short-wavelength UV light. Products were identified by comparison of chromatograms of culture extracts to those of authentic commercial standards.

Table 1
Biomass Production by Paecilomyces During Growth on Inorganic Sulfur Compounds

Substrate	Oxidation state	Cell mass[1] (mg/culture)
Unsupplemented (control)	N/A	48.5
Sulfate	6^+	225.6
Sulfite	4^+	354.0
Thiosulfate	2^+	175.2
Elemental sulfur[2]	0	103.0
Sulfide	2^-	117.5

[1] Average of two cultures

[2] "Flowers of sulfur"

Reagents

The following compounds were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI); figures in parentheses indicate their purity: benzenesulfonic acid (90%), biphenyl (99%), DBT (98%), DBT sulfone (97%), dibenzyl disulfide (98%), dibenzyl sulfide (98%), 2,2'-dihydroxybiphenyl (99%), diphenyl disulfide (99%), diphenyl sulfide (98%), diphenyl sulfone (97%), ethyl phenyl sulfide (97%), thianthrene (97%), and thiophenol (99+%). All other chemicals were of reagent grade.

RESULTS

Utilization of Inorganic Sulfur Compounds

An initial investigation was designed to assess *Paecilomyces'* ability to utilize a range of inorganic sulfur substrates differing with respect to oxidation state. These and subsequent experiments were confounded by the organism's efficient scavenging of sulfur contaminants present in the unsupplemented medium. Approximately one-fourth as much biomass was produced in the unsupplemented medium as was produced in medium containing 2 m*M* sulfate (Table 1). However, all other forms of inorganic sulfur tested—sulfite, thiosulfate, elemental sulfur, and sulfide—supported significantly more growth than that observed in the unsupplemented culture as well. Cultures containing sulfite produced the greatest amount of biomass. These results suggest that *Paecilomyces* is quite versatile with respect to its sulfur metabolism, and is competent to utilize partially reduced sulfur. Growth on elemental sulfur in particular indicates an appar-

ent ability to utilize insoluble substrates. In these cultures, the growing mycelium engulfed the sulfur particles, which acted as nuclei for pellet formation. Ability to use elemental sulfur, coupled with this growth mode, suggests the existence of extracellular or cell surface-associated sulfur metabolizing enzymes.

Utilization of Organic Sulfur Compounds

Subsequent experiments were designed to determine whether organic sulfur compounds were utilized as growth substrates (i.e., carbon and/or sulfur sources). *Paecilomyces* was clearly incapable of utilizing either DBT and thianthrene as the sole carbon source when the test compound was added at 1% final concentration in medium containing 2 m*M* sulfate (data not shown). Growth occurred only in the presence of an easily metabolizable carbon source, such as maltose. The amounts of biomass produced during growth in medium containing 5% maltose when either DBT or thianthrene was provided as the primary sulfur source were equivalent, within the limits of experimental error, to that produced in unsupplemented medium containing DMF and Tween-80 (data not shown). Neither DBT nor thianthrene inhibited growth of *Paecilomyces* in standard Czapek's medium (containing 1% maltose and 2 m*M* sulfate) when added at 1–2 m*M* concentration, suggesting that the these compounds are not toxic. The absence of significant growth in cultures provided with the test compounds as sulfur sources thus indicates that these compounds are not utilized.

The apparent requirement for a cosubstrate for the degradation of DBT and thianthrene suggested that activity against organic sulfur compounds would be generally promoted by high maltose concentrations. Indeed, preliminary results suggested that *Paecilomyces* generated greater amounts of products from DBT when grown on 5% maltose than when grown on 1% maltose (data not shown). For these reasons, results of experiments conducted with cultures containing 5% maltose are presented below. Under these (nitrogen-limiting) experimental conditions, biomass production ceased within 2.5 d of incubation.

Degradation of Organic Sulfur Compounds

Considerable thought was given to the methodology for the analysis of the experimental culture extracts produced in the course of these studies. An extensive thermal degradation of DBT and its microbial metabolites during GC/MS analysis has been described elsewhere (*10,16*), and was confirmed in the present work. Similarly, organic sulfides and disulfides were found to generate anomalous products during GC analysis. The GC/MS analytical results presented below, therefore, describe only those products unique to biological extracts. Ambient-temperature TLC analysis was performed to corroborate product identity when the appropriate

Table 2
Products Formed by Paecilomyces from DBT[1]

	R_f values				
Standards:					
DMF	----	----	0.62	----	----
DBT	0.88	----	0.62	----	----
DBT sulfone	----	0.69	0.62	----	----
2,2'-biphenol	----	----	0.61	0.50	----
Uninoculated controls:[2]					
DBT	0.89	----	0.63	----	----
Experimental cultures:					
Control (DMF only)[3]	----	----	0.62	----	----
DBT (3-d culture)	0.85	----	0.62	----	0.38
DBT (10-d culture)	0.89	0.70	0.63	----	0.38
DBT (30-d culture)	0.90	----	0.62	0.49	----

[1] Thin layer chromatographic analysis of CH_2Cl_2 extracts of cultures incubated with DBT. Solvent = 1-heptane:acetone, 1:1. DMF, which was present in all cultures, was used as an internal standard.

[2] Results are presented for a 3-d culture; similar results were obtained through day 30.

[3] Results are presented for a 10-d culture; similar results were obtained through day 30.

authentic standards were available, and indeed, was the preferred method for the analysis of DBT culture extracts. TLC analysis was also used to identify underivatized sulfonic acids and other acidic products, which would not be detected by the GC/MS system used here. (Phenols were detectable by GC analysis.)

The fate of DBT in cultures was studied over the course of a 30-d incubation. TLC analysis of methylene chloride extracts of these cultures showed the appearance of an unidentified metabolite on day 3 (R_f=0.38) and of a compound coeluting with DBT sulfone (R_f=0.70) on day 10 (Table 2). These compounds disappeared from cultures on further incubation. By day 30, a compound coeluting with 2,2'-dihydroxybiphenyl (R_f=0.49)

had accumulated within cultures. Only DBT and diphenyl were detected through GC/MS analysis of these extracts (i.e., there was no evidence for ring-cleavage pathway metabolites, such as 1,2-dihydroxy-DBT or 3-hydroxy-2-formyl benzothiophene). (Biphenyl, presumably a thermal degradation product, also appeared in gas chromatograms of the authentic DBT standard. Biphenyl was not detected by TLC of the DBT standard, nor did it coelute with the unidentified bioproduct.) Gas chromatographic analysis of the 2-butanol extracts of these cultures revealed no compounds other than those mentioned above. Analysis of these extracts by TLC indicated the presence of a presumably acidic product that was not identified. No other products (including single-ring compounds such as catechol) arising from further metabolism of 2,2′-dihydroxybiphenyl were detected by TLC or GC. Cultures to which DBT sulfone was added at the time of inoculation produced 2,2′-dihydroxybiphenyl within 10 d; no further metabolism was observed over a total of 30 d (TLC analyses). Preliminary results regarding the fate of 2,2′-dihydroxybiphenyl in cultures when added at the time of inoculation were inconclusive, although acidic products were apparently formed (TLC analysis).

Analysis by GC/MS was performed on the methylene chloride extracts of cultures incubated for 7 d with organic sulfide test compounds. Cultures containing ethyl phenyl sulfide generated the corresponding sulfone as the major product, plus di-(2′-hydroxyethyl)-benzenes. Diphenyl sulfide cultures yielded diphenyl sulfone (major product) and diphenyl disulfide. These products were unique to extracts of inoculated cultures (i.e., were clearly of biological origin). Cultures incubated with dibenzyl sulfide generated a variety of unique products, including substituted monoaromatic compounds (Fig. 3). Analysis of methylene chloride extracts of thianthrene cultures by GC/MS indicated the presence of diphenyl sulfide. Analysis of the same extract by TLC provided some corroborating evidence, and indicated the presence of other, lower-mol-wt and/or more polar products as well (Table 3). Benzenesulfonic acid was not detected. Analysis by TLC of the 2-butanol extracts of ethyl phenyl sulfide, diphenyl sulfide, and thianthrene cultures indicated that benzenesulfonic acid was not formed.

Analysis by GC/MS was also performed on methylene chloride extracts of 7-d cultures containing organic disulfides. Diphenyl disulfide generated thiophenol plus an alkyl- or alkenyl-substituted thiophenol. Dibenzyl disulfide generated benzy thiol plus alkylbenzenes. Analysis by TLC of the 2-butanol extract of diphenyl disulfide indicated that benzenesulfonic acid was not formed.

Analysis of 2-butanol extracts of cultures containing thiophenol by GC/MS indicated that the test compound completely disappeared from cultures during a 7-d incubation. Products were not detected by GC. Analysis by TLC revealed the presence of a presumably acidic product that did not coelute with benzenesulfonic acid and was thus not identified.

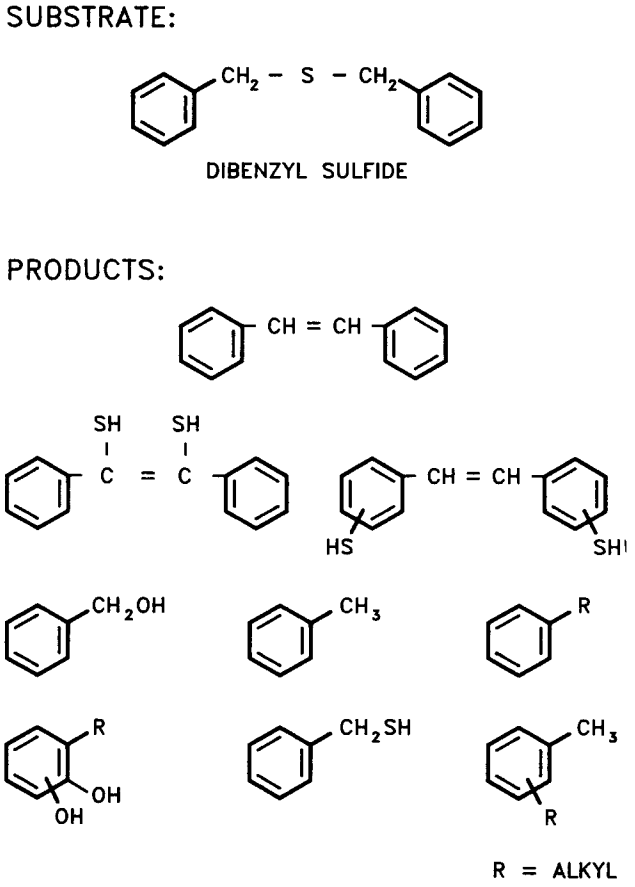

Fig. 3. Products formed by *Paecilomyces* from dibenzyl sulfide.

DISCUSSION

This work was designed to assess the ability of *Paecilomyces* to degrade organic sulfur-containing coal model compounds under cultural and environmental conditions shown previously to support the production of coal-solubilizing activity (*14,15*). These experiments were qualitative in nature: mass balances were not attempted, although 22 to 28% conversion of the substrates was commonly observed over a 7-d incubation (GC analysis).

Results presented in this paper suggest that *Paecilomyces* is capable of transforming DBT and other organic sulfur compounds via cometabolism. Oxidation of DBT by *Pseudomonas* spp. was shown elsewhere to require the presence of a cosubstrate (*8,17*); but in contrast to the present work, an easily metabolizable carbohydrate (glucose) inhibited activity. Carbon catabolite repression was apparently not a factor in the regulation of the degradative activities produced by the nitrogen-limited cultures used here.

Table 3
Products Formed by Paecilomyces from Thianthrene[1]

	R_f values				
Standards:					
DMF	----	----	----	0.60	----
Thianthrene	----	0.90	----	0.62	----
Phenyl sulfide	0.95	----	----	0.62	----
Experimental cultures:[2]					
Control (DMF only)	----	----	----	0.62	----
Thianthrene[3]	0.93	0.90	0.74	0.61	0.45

[1] Thin layer chromatographic analysis of CH_2Cl_2 extracts of cultures incubated with thianthrene. Solvent = 1 heptane:acetone, 1:1. DMF, which was present in all cultures, was used as an internal standard.

[2] Results are presented for a 10-d-old cultures. Thianthrene was stable in uninoculated controls through day 10.

A DBT degradation product from *Paecilomyces* was first detected immediately after the end of the active growth phase. Another product, DBT sulfone, was first detected several days later. This kinetic profile suggests either that the unknown product was a precursor of DBT sulfone, or that two DBT transformation pathways are operative in this organism. The former possibility suggests that this unidentified product was DBT sulfoxide. The unidentified product was not detectable by GC/MS, and presumably degraded at high temperatures. Sulfoxides are known to be similarly thermolabile (*16*). The latter possibility suggests a potentially biphasic fungal attack on DBT. Mormile and Atlas (*10*) described a *Pseudomonas* sp. that exhibited both a growth-related ring-destructive activity and a secondary metabolic, or idiophasic, sulfur-oxidizing activity toward DBT. These authors were able to identify intermediates in both pathways by GC/MS analysis. They also detected another product by TLC that reverted to DBT during GC/MS analysis. This product, which was red, is probably the ring-cleavage product described elsewhere (*9*). The unidentified product generated in the present work was similarly thermolabile, but a red color was not detected in cultures during the course of the 30-d incubation. These observations suggest that this unknown metabolite is not the same product.

The presence of the unidentified metabolite makes it impossible to rule out the possibility of a ring-destructive or other DBT transformation activity. However, the absence of evidence of other, thermostable metabolites arising from the ring-destructive pathway suggests that metabolic flux in this organism proceeds primarily via sulfur oxidation. The idiophasic transformation of DBT (or its metabolite) apparently proceeded via direct oxidative (oxygenative) attack at the sulfur atom with formation of the corresponding sulfone. That compound was ultimately cleaved to form 2,2'-dihydroxybiphenyl, presumably with release of free sulfate.

Results discussed above suggest that attack on organic sulfides, like that on DBT, would proceed at least in part via a sulfur-specific (oxygenative) oxidation. Indeed, ethyl phenyl sulfide and diphenyl sulfide were transformed to the corresponding sulfones. But neither sulfonic acids nor phenolic compounds, which would be predicted to arise from sulfate release, were observed over the course of this 7-d incubation. It is not known whether these more-oxidized products ultimately would have appeared. Dibenzyl sulfone was not detected, although the corresponding cleavage product (benzyl alcohol) did appear in cultures (although not necessarily by the proposed pathway; *see below*).

The variety of products generated from nonheterocyclic organic sulfides in general, and dibenzyl sulfide in particular, suggests an underlying mechanism for degradation of organic sulfur compounds by this organism, namely, a sulfur-specific attack by a biological catalyst with generation of free radicals. A possible reaction mechanism would involve an initial one-electron oxidation of the sulfur atom with formation of a thiyl radical. A fairly straightforward subsequent cleavage and condensation would result in the formation of a disulfide, as observed in the case of diphenyl sulfide. However, the thiyl radical would also be expected to undergo rearrangement to a variety of resonance forms. Localization of the unpaired electron on an adjacent carbon atom would yield aryl and/or alkyl radicals that would be free to undergo rapid condensations, with formation of substituted benzenes. Benzylic radicals are particularly stable (*16*), perhaps explaining the proliferation of products from dibenzyl sulfide. Thiyl radical-mediated oxidations of this type have been implicated in the biological degradation of nonphenolic lignin model compounds by a manganese peroxidase of *P. chrysosporium* (*18*). The ability of this *Paecilomyces* isolate to produce peroxide or peroxidases has not been studied.

In the present system, thianthrene was transformed to diphenyl sulfide. The mechanism underlying this activity is not known. However, this apparent attack on only one of the two sulfur atoms is similar to that observed during treatment of thianthrene with *P. chrysosporium* lignin peroxidase (*11*). Thianthrene was oxidized in vitro to its corresponding monooxide via a sulfur-specific, oxygenative attack. However, the formation of cation radicals, which are characteristically produced during reactions with this enzyme (*19*), was not detected during the reaction with

thianthrene. The relevance of this observation to the involvement of thiyl radicals in fungal oxidation of organic sulfur compounds, as proposed here, is not known.

Transformation of organic disulfides in cultures appeared to be somewhat less complex than those of sulfides. Thiol formation is consistent with a simple cleavage across the sulfur bond; the underlying mechanism, however, cannot be determined from this work. The appearance of substituted alkylbenzenes (particularly in the case of dibenzyl disulfide) suggests the involvement of thiyl radicals as described above.

The present work provides little insight into the kinetics of the attack on sulfur by this organism or the identity of the catalyst(s) involved. Production and breakdown of DBT sulfone were clearly independent of growth, as was further metabolism of the unidentified material. This behavior is consistent with prior reports that described the production of an oxidative coal solubilizing activity by fungi as a secondary metabolic or idiophasic function (*12,20*). *Paecilomyces* was shown previously in this laboratory to carry out an oxidative degradation of soluble coal components (*15*). This activity occurred slowly over a prolonged (120-d) incubation period that extended well past the active growth phase. It is possible that some of the same oxidative, idiophasic catalysts involved in that degradative activity contribute to the transformation of DBT and its metabolites. A similar hypothesis would explain too the persistence of thiophenol (produced from diphenyl disulfide) in cultures, a somewhat surprising event in light of the apparent disappearance of thiophenol from cultures when added at the time of inoculation. It is possible that thiophenol is degraded only by growing cells, and that the attack on diphenyl sulfide is strictly idiophasic. The apparent transformation of 2,2′-dihydroxybiphenyl within growing, but not idiophasic, cultures suggests a similar temporal compartmentalization of degradative activities.

In the present work, oxygenative and nonoxygenative oxidation reactions contributing to the transformation of nonheterocyclic organic sulfides compounds appeared to proceed in tandem. Factors controlling the relative importance of oxygenative and nonoxygenative mechanisms were not determined. The cultures described here were grown in submerged mode in the absence of forced aeration. It is possible that increased accessibility to oxygen would promote oxygenative activity leading to release of free sulfate ion. Oxygen has been proposed elsewhere to act as a scavenger of thiyl radicals, with formation of sulfonated products (*18*). Indeed, the sulfones observed in this work may have been formed in this manner.

The results of this work suggest that *Paecilomyces* has potential utility in the desulfurization of coal-related compounds. This organism may form the basis for the design of a combined process for solubilization of low-rank coal, and for the cleaning of the biosolubilized product *in situ*. An alternative application would be in the desulfurization of high-rank coal, which is not solubilized by *Paecilomyces*. This organism's demonstrated

ability to catalyze a surface attack on coal during solubilization, coupled with the current findings, suggest that it may catalyze sulfur removal from the surface of finely ground coal particles. *Paecilomyces* and other sulfur-oxidizing organisms may be particularly active at the interface between organic and inorganic regions within coal, where "immature," relatively labile sulfur-containing structures are thought to be localized (*21*). A sulfur-specific attack designed to preserve the substrate's carbon content or caloric value would require operating conditions that discourage ring cleavage reactions. The use of pregrown biomass may achieve this end. The relative yields of oxygenated and nonoxygenated products, and of sulfur-containing and nonsulfur-containing products, will be important determinants of the practical utility of coal desulfurization processes based on this organism.

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