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Use of Fungi in Biodegradation

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In nature, fungi do much of the dirty work. They are particularly efficient at degrading the major plant polymers, cellulose and lignin, but they also decompose a huge array of other organic molecules including waxes, rubber, feathers, insect cuticles, and animal flesh. Although industrial microbiologists regularly harness fungal metabolism for brewing, baking, cheese preparation, and for production of antibiotics, commercial enzymes, and a number of commodity chemicals, fungi are best known for their dirty work. They spoil our foods, blight our crops, rot our buildings, contaminate our petri dishes, and cause some rather loathsome diseases. Paradoxically, despite this notoriety, the use of fungi in bioremediation has been limited compared to that of bacteria. Here we present a brief introduction to fungal taxonomy and mycological techniques, introduce methods for isolating fungi and for growing them in the laboratory, define some important terms, review examples of the successful applications of fungal organisms and enzymes for biodegradation, and point out the advantages and disadvantages of fungi as agents of bioremediation.

A LITTLE TAXONOMY

Like many other higher-order taxonomic units, the term "fungus" is difficult to define. It embraces a large group of nonphotosynthetic lower eukaryotes once considered part of the plant kingdom and later afforded status in their own kingdom, the "Fifth Kingdom," on the basis of their characteristic absorptive mode of nutrition (102, 117, 168). Traditionally, the Myxomycota, or slime molds, and the Eumycota, or "true fungi," comprised the two major subdivisions within this fungal kingdom. A plasmodium or pseudoplasmodium characterized the Myxomycota, a group which included well-known genera such as *Dictyostelium* and *Physarum*, while in the Eumycota the assimilative phase was usually filamentous or yeast-like. More recently, classification based on evolutionary relationships (i.e., phylogenetic classification) has led to a realization that the organisms that have traditionally been called fungi, on the basis of shared nutritional modes and morphological characters, do not represent a monophyletic lineage. Derived primarily from data on small-ribosomal-subunit (rDNA) sequence analysis, the assemblage of traditional fungi is now placed in three groups: the kingdom Fungi, the kingdom Stramenopila, and four protist phyla. In this classification, the kingdom Fungi encompasses four phyla: Chytridiomycota, Zygomycota, Ascomycota, and Basidiomycota (18, 26). The Stramenopila encompasses three phyla: Oomycota, Hyphochytriomycota, and Labyrinthulomycota. From the perspective of research on fungal degradation, most of the species of interest are in the kingdom Fungi.

In filamentous forms, the individual thread-like cells are called hyphae. A fungal colony, or portion of a colony, composed of many hyphae together is called a mycelium. The filamentous/mycelial growth form poses problems in determining the size of a single organism and in measuring the growth of fungi. In the older literature, the term "thallus" is often used to describe macroscopic mycelial formations.

Fungal taxonomy is based on reproductive morphology, which consists of both meiotic and mitotic spore-bearing structures. Both sexual and asexual spores are typically made in vast numbers. Many fungi have more than one morphologically distinct spore type at different phases in their life cycles. Further complicating this fungal pleomorphism is the fact that some fungi exist as either yeast or filamentous forms depending on the environmental milieu, a phenomenon called dimorphism, best known from medically important species.

Like other eukaryotes, fungi have nuclei, mitochondria, 80S ribosomes, and chromosomes. Fungal cells may be haploid or diploid; the nuclei within a mycelium may all be genetically identical (monokaryotic) or may be a mixture of different genetic types (heterokaryotic). Basidiomycetes often have a special form of heterokaryon called a dikaryon. Although many fungi are microscopic, the best-known species form macroscopic fruiting structures such as mushrooms and truffles. Fungi are ubiquitous in terrestrial environments, and many fungi are capable of growing in environments hostile to most other forms of life (92). For example, fungi are the only eukaryotes that include members with thermophilic (60 to 62°C) optimal growth temperatures (158).

In summary, the broadly understood concept of fungi comprises a polyphyletic group of eukaryotic, heterotrophic organisms that absorb their food. Mycology texts such as those by Alexopoulos, Mims, and Blackwell (10), Ross (143), Moore-Landecker (124), and Carlile and Watkinson (30) are helpful introductions to this economically impor-

tant group. Another useful tool is *Ainsworth & Bisby's Dictionary of the Fungi* (83). General taxonomic principles, as well as a guide to the sometimes arcane principles of fungal nomenclature, which are governed by the International Code of Botanical Nomenclature, are presented by Hawksworth (79). The last volumes (in two parts) of the classic series, *The Fungi: an Advanced Treatise*, provide comprehensive taxonomic coverage (2, 3), while the first volumes (4–6) give an almost encyclopedic review of the classical mycological literature. Another useful resource is the multivolume series entitled *The Mycota, an Encyclopedia of Fungi* (50). Volume VII addresses fungal classification and taxonomy (120).

ACQUISITION, CARE, AND FEEDING OF FUNGI

Filamentous fungi have more described species than any other group of microorganisms, with about 80,000 already named and approximately 1,800 new species published each year. As with bacteria, it is believed that only a small proportion of extant species are known to science. The total number of fungal species, both known and unknown, has been estimated at more than 1.5 million (80, 82).

About 170,000 pure strains of fungi are maintained in culture collections internationally, representing an estimated 7,000 different species (81). Information about these resources can be accessed through the *World Directory of Collections of Cultures of Microorganisms* (156). One of the oldest and largest fungal collections is the Centraalburear voor Schemmelcultures in the Netherlands. The major collection in the United States is the American Type Culture Collection (98). In addition, the collection at the Northern Regional Research Laboratory of the U.S. Department of Agriculture in Peoria, Ill., houses a large number of economically important fungi, and the Forest Products Laboratory in Madison, Wis., holds a major collection of wood-rotting species. Major culture collections maintain Web sites with information about holdings and instructions about ordering. As with bacterial and viral strains, a fee is usually charged for obtaining cultures.

In the laboratory, fungi and bacteria are treated similarly. They can be grown on agar media in petri plates or in liquid broths. Depending on the nutritional requirements of the individual species, either a complex or defined medium is used. Many fungi prefer media with acidic pH. On petri plates, molds are readily distinguished from most bacteria because they usually form dry aerial colonies that may be brightly colored due to the pigmentation of their spores. With the naked eye, yeast colonies are not so easily distinguished from bacteria, but under the light microscope, their large size, compared to any run-of-the-mill prokaryote, is easy to discern. Recipes for common and specialty media for cultivating fungi are available in *Mycology Guidebook* (155) and *Handbook of Microbiological Media* (15). In isolating fungi from nature, antibiotics, such as streptomycin, or other bacterial growth inhibitors, such as rose bengal, are frequently added to media, thereby selectively enhancing the growth of fungi (119).

When isolating culturable microorganisms from natural substrates, it is relatively easy to obtain a colony count based on the unicells of typical bacteria. Sampling for fungi is much harder due to their filamentous growth habit. There may be a large mass of fungal mycelium present, but the methods adapted from bacteriology may not detect it. Moreover, dormant fungal spores may produce numerous colonies while thriving nonsporulating colonies may not be recovered at all. In liquid shake culture, many filamentous fungi form pellets, thus making direct turbidity assays impractical and making dry weights the most commonly used measure. In batch culture, the synthesis of many fungal products and enzymes is not correlated with growth but is triggered by the limitation of an essential nutrient. The terms "trophophase" and "idiophase," roughly comparable to bacterial log phase and stationary phase, respectively, have been used to describe filamentous growth. Both secondary-metabolite production and the ligninolytic-enzyme production are correlated with idiophase.

Enrichment cultures are a classical microbiological technique, commonly used for finding a specific microbe to degrade a certain toxic waste. Enrichment cultures favor the growth of a particular species based on its nutritional requirements. In the most common application of this method, aliquots of water, soil, leaf litter, or other mixed inocula are placed into a medium containing the targeted compound as the sole carbon source. Only organisms with degradative ability will grow. In liquid culture, competition for the substrate will lead to enrichment of the microbial strain that is able to grow fastest. On petri plates, colonies representing many species are usually isolated; these are then subcultured and tested further. With few exceptions, this approach leads to the isolation of bacteria. In general, fungi are slower growing and produce fewer propagules than do bacteria. In addition, fungi are less likely than bacteria to have the capacity to use xenobiotics as sole carbon sources. Many fungi need a supplemental carbon source to sustain growth, i.e., their degradative potential is cometabolic.

Thus, the key to successful isolation of fungi for xenobiotic degradation is twofold: (i) the recognition that fungi are easily outgrown by bacteria and (ii) the recognition that they produce many potent biodegradative enzymes capable of degrading toxic pollutants yet do not use these breakdown products to sustain growth. To successfully isolate fungi with potential for bioremediation, it is necessary to impose imaginative enrichment conditions, including the careful selection of supplementary carbon sources and bacteriostatic agents.

Serving as a manual to both methods and references, volume 4 of *Methods in Microbiology* (24) is specifically devoted to distinguishing mycological and bacteriological perspectives. Specialized techniques for collecting, isolating, cultivating, manipulating, and preserving fungi are given. Individual chapters are devoted to soil fungi, air sampling, and aquatic organisms. Although several decades old, this book is still a wonderful resource. See also *Techniques in Microbial Ecology* (74), *Mycology Guidebook* (155), and chapters 6, 35, and 49 for guidance on the special handling of fungi. Other valuable references concentrating on applied mycology are Arora et al. (13), Smith and Berry (151, 152) and Smith et al. (153).

DEFINITIONS AND DISTINCTIONS

Biodegradation, mineralization, bioremediation, biodeterioration, biotransformation, cometabolism, and bioaccumulation are terms not always used with appropriate sensitivity to their subtle differences in meaning. The special role played by fungi in nature and in the human exploitation of their often unique metabolism can be clarified

by thinking about the distinctions implied in this ecological lexicon.

"Biodegradation" is the biologically mediated breakdown of chemical compounds. It is an umbrella term, encompassing most of the other jargon addressed in this section, and generally implies a series of biochemical reactions. When biodegradation is complete, the process is called "mineralization," i.e., the total breakdown of organic molecules into water, CO_2, and/or other inorganic end products. Not all authors are careful to distinguish between degrees of biodegradation; some use the term to describe almost any biologically mediated change in a substrate, and others use it to describe mineralization (9).

"Biotransformation," a word often used synonymously with "bioconversion," usually refers to a single step in a biochemical pathway, in which a molecule (the precursor) is catalytically converted into a different molecule (the product). Many biotransformations in sequence constitute a metabolic pathway. Industrial processes frequently incorporate biotransformation reactions. A famous example is in the manufacture of steroids for birth control pills (141). When ecologists describe biotransformation, the environmental status of the transformed product is a primary consideration. Is it more water soluble and thus more easily excreted by the cell? Is it less toxic? Is it more hazardous than its precursor? When relatively innocuous precursors are converted into more toxic products, the process is called "activation." The metabolic activation of mercury is a well-known example of a biotransformation with malign environmental impact.

"Biodeterioration" and "bioremediation" are the two aspects of biodegradation with an anthropomorphic emphasis. Biodeterioration is the breakdown of economically useful substances. Often the term is used narrowly to refer to the deterioration of substances that are normally resistant to biological attack such as metals, plastics, drugs, cosmetics, paintings, sculpture, wood products, electrical equipment, fuels and oils, and other economically valuable objects (142).

In bioremediation, biological systems are used to transform and/or degrade toxic compounds or otherwise render them harmless. Bioremediation can involve indigenous microbial populations with or without nutrient supplementation, or it can involve inoculation of exogenous organisms into the site. When exogenous organisms are added, the process is called "bioaugmentation." In either case, the goal is to disarm noxious chemicals without the formation of new toxins.

"Bioaccumulation," sometimes used loosely synonymously with "biosorption," is the concentration of substances without any metabolic transformation. Both living and dead cells may be involved. Bioaccumulation techniques can be used to concentrate metals such as copper, lead, silver, uranium, and certain radionuclides from aqueous environments, and the resultant loaded biomass can be recycled or contained.

The term "cometabolism" is used in two senses. Usually, it describes the situation where an organism is able to biotransform a substrate but is unable to grow on it. As defined by Horvath, "Co-metabolism refers to any oxidation of substances without utilization of the energy derived from the oxidation to support microbial growth" (88). The phenomenon has also been called "cooxidation" and "gratuitous" or "fortuitous" metabolism. Many biochemists dislike the term (67, 89). Nevertheless, it has become well entrenched in the literature.

A second meaning of cometabolism is to describe the degradation of a given compound by the combined efforts of several organisms pooling their biochemical resources for mutual efforts (41).

THE FUNGAL WAY OF DEGRADATION

The organisms known as fungi, encompassing both Fungi and Stramenopila, share a unique nutritional strategy, i.e., their cells secrete extracellular enzymes which break down potential food sources, which are then absorbed back into the fungal colony. This way of life means that any discussion of fungal biodegradation must cover an extraordinary amount of catalytic capability. The decomposition of lignocellulose is probably the single most important degradative event in the Earth's carbon cycle. The utilization and transformation of the dead remains of other organisms is essential to the Earth's economy. An enormous ecological literature exists on the role of fungi as primary and secondary decomposers in these classic "cycles" of nature (see, for example, references 8, 31, 36, and 60).

From the human perspective, the power of fungal enzymes is Janus-faced. Molds destroy more food than any other group of microorganisms. They damage standing timber, finished wood products, fibers, and a wide range of noncellulosic products such as plastics, fuels, paints, glues, drugs, and other human artifacts (48, 142). On the other hand, many of the oldest biotechnological practices are also based on fungal catalytic power: baking, brewing, wine fermentation, production of certain cheeses, and the koji process are ancient examples of the way humans have employed fungi for their own benefit. In the 20th century, numerous hydrolytic enzymes involved in the degradation of relatively simple biopolymers such as starch and protein have been purified, characterized, and utilized within industrial settings. These include fungal amylases, glucoamylases, lipases, pectinases, and proteases (see references 19 and 22 for reviews). Fungal cellulases provide a good example of the contrasting faces of a single enzymatic capability. During World War II, research by the U.S. Army on the microbial destruction of military clothing and tents led to the characterization of the cellulolytic mold *Trichoderma reesei*. Continuing research on *T. reesei* identified a complete set of cellulase enzymes required for the breakdown of cellulose to glucose (128, 139). These enzymes now promise the potential of converting waste cellulosics into foods for our burgeoning population and have been the subject of intense molecular biology research (51, 123). Although cellulase-produced glucose is not yet economically competitive, another traditional fungal process is: mushroom cultivation on lignocellulose (34, 154). These and some other examples of economically advantageous uses of fungal biodegradation are displayed in Table 1.

Fungi are also good at bioaccumulation of metals. Many species can adsorb cadmium, copper, lead, mercury, and zinc onto their mycelium and spores. Sometimes the walls of dead fungi bind better than living ones. Systems using *Rhizopus arrhizus* have been developed for treating uranium and thorium (161). Spent fungal biomass from industrial fermentations is an available resource for the concentration of heavy-metal contamination (62, 63, 144).

What about fungal degradation of pollutants and toxic wastes? In some cases, traditional methods are being adopted for contemporary needs. For example, composting has been used to treat both pesticides (59) and munitions

TABLE 1 Economically beneficial examples of fungal degradation

Process	Substrate	Species	Representative citations
Composting	Straw, manure, agricultural waste, bark	Consortia of bacteria and fungi, usually uncharacterized	21, 55
Mushroom cultivation	Lignocellulose, animal manure	*Agaricus bisporus*	34, 84
	Straw, sawdust	*Pleurotus ostreatus* (oyster mushroom)	154
	Wood logs	*Lentinus odoides* (shiitake)	154
Single-cell protein production	Alkanes	Yeasts, e.g., *Candida tropicalis*	42, 100
	Brewery wastes, molasses	*Saccharomyces cerevisiae*, *S. carlsbergensis*	114
	Sulfite waste liquid	*Candida utilis*, *Paecilomyces varioti*	114
Solid-waste treatment	Sludge/sewage	Consortia of bacteria and fungi, usually uncharacterized	37, 39
	Pulp and paper mill effluent	*Coriolus versicolor*, *Phanerochaete chrysosporium*	106, 107
Wastewater treatment	Distillery waste	Yeasts, especially *Candida utilis*	61
	Kraft bleaching effluent	*Phanerochaete chrysosporium*	49
	Tannery effluent	*Aspergillus*, *Penicillium*	127

wastes (169). There is also rather a lot of descriptive biochemistry concerning the ability of various fungi and their enzymes to biotransform pesticides (see reference 138 for enzymes and reference 150 for lists of specific compounds and organisms), but, to date, the most sophisticated fungal approaches to environmental clean-up have grown out of prior research on degradation of petroleum hydrocarbons (16, 32, 66) and on the adaptation of research on fungal treatment of lignocellulytic wastes in the pulp and paper industry (106, 107, 109).

The ability to grow on petroleum hydrocarbons is widespread among the fungi (14, 33, 66). Jet crashes caused by blocked fuel lines due to the growth of *Cladosporium resinae*, first reported during World War II, are among the more dramatic negative consequences of the ability of fungi to thrive in extreme habitats (113). Considerable information is available about the mycological flora associated with marine petroleum spills (33). On the industrial side, the years of research on single-cell protein, instituted with the goal of turning petroleum hydrocarbons into feed, have paid off in the study of the enzymatic mechanisms used by yeasts and other microorganisms in the biodegradation of petroleum wastes for environmental remediation (100, 140). Cytochrome P-450s are mixed-function oxidases (monooxygenases), derived from a superfamily of genes, which are involved in many steps of petroleum degradation and in the biotransformation of a variety of environmental pollutants (166). Both detoxification and activation are associated with the action of P-450. Fungal monooxygenases are more similar to mammalian than to bacterial cytochromes; Sariaslani (146) has presented a particularly thorough review of these enzymes, and Kellner et al. (101) have discussed their use in bioremediation. Extensive biochemical and genetic data are available for several yeasts; there is also a large literature surrounding the aseptate filamentous species *Cunninghamella elegans* (33, 66).

Similarly, and to an even greater extent, research on pulp waste treatment, such as the decolorization of effluent from kraft pulp mills, and the subsequent mushrooming of research on white rot fungi have shown the power of wood-decaying species against a surprisingly large battery of environmental contaminants (29, 58). Recent advances in the use of fungi in environmental remediation and biotechnology have been summarized by Paszczynski and Crawford (132).

PHANEROCHAETE CHRYSOSPORIUM

Phanerochaete chrysosporium is a higher basidiomycete belonging to the white rot group of fungi. *P. chrysosporium* is the best studied of the ligninolytic fungi, a group whose natural habitat is forest litter and rotting wood. White rot fungi are unique among eukaryotes in having evolved nonspecific mechanisms for degrading lignin.

Lignin is unlike many natural polymers in that it consists of irregular phenylpropanoid units linked by nonhydrolyzable carbon-carbon and ether bonds. Lignin contains chiral carbons in both the L and D configuration, and this stereo irregularity renders it still more resistant to attack by most microorganisms. Nevertheless, many extracellular ligninolytic enzymes produced by white rot fungi can catalyze the breakdown of lignin.

Under conditions of nitrogen, sulfur, and/or carbon deprivation, *P. chrysosporium* produces families of ligninolytic enzymes (54, 68, 160) including lignin peroxidase (LiP) (EC 1.11.1.12) (160) and manganese-dependent peroxidase (MnP) (EC 1.11.1.13) (69). The peroxidases use hydrogen peroxide generated by glyoxal oxidase, glucose oxidase, and cellobiose oxidase to promote the oxidation of lignin to free radicals that then undergo spontaneous reactions with oxygen or water, which leads to depolymerization. The depolymerization of lignin by nonspecific extracellular peroxidases is sometimes called enzymatic combustion (108). Both LiP and MnP are encoded by families of structurally related genes that have been cloned and sequenced (for reviews, see references 7, 25, 40, and 87). These genes are differentially regulated in response to a variety of environmental signals, especially starvation (91).

During the 1980s it became apparent that *P. chrysosporium*, in addition to degrading lignin, is capable of

degrading a wide variety of xenobiotics. Polyaromatic hydrocarbons, chlorinated phenols, nitroaromatics, dyes, and many other environmental toxins have been biotransformed or mineralized by *P. chrysosporium*, sometimes in complex mixtures of xenobiotics (Table 2). The ability to degrade such a broad spectrum of highly toxic and generally recalcitrant substrates is unusual for a single species. It is often assumed that this broad-spectrum xenobiotic biodegradation is effected by the same extracellular enzymes used in lignin degradation. In addition, a variety of other factors are thought to contribute, such as intracellular enzymes (e.g., reductase, methyltransferases, and cytochrome P-450 oxygenases), "plasma membrane potentials," and bioabsorption onto mycelia (12, 99, 163, 164). Postulated mechanisms used by the white rot fungi to degrade pollutants have been summarized by Barr and Aust (17).

In the laboratory, most of the successful mineralization experiments have been conducted under ligninolytic conditions using whole cells in liquid culture. Moreover, purified preparations of lignin peroxidases are capable of oxidizing a variety of the xenobiotics known to be mineralized by whole cell cultures of *P. chrysosporium* (e.g., 2,4-dinitrotoluene, lignite, polyaromatics, pentachlorophenols, and dichlorodibenzo-*p*-dioxin, pyrene), although they are not involved in others (e.g., DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane]) (85, 86, 110). On the other hand, many questions remain unanswered. In one study, the disappearance of 2,4,6-trinitrotoluene (TNT) began prior to the onset of ligninase activity, while secondary products were formed after the appearance of LiP and MnP (78). In another study, *P. chrysosporium* removed phenanthrene under both ligninolytic and nonligninolytic conditions (43).

To recapitulate, among filamentous fungi, *P. chrysosporium* has become a model system for studying xenobiotic degradation. A critical assessment of its potential as a bioremediation tool has been given by Paszczynski and Crawford (131), who point out that the field applications have been unpredictable and that even under ideal laboratory conditions many contaminants are only partially broken down. In some cases, environmentally undesirable breakdown products are formed. In addition, many theoretical questions remain about the biochemical activities of *P. chrysosporium* in nature and the relationship between the enzymes of lignin degradation and the mechanisms of xenobiotic removal. In practice, the application of *P. chrysosporium* to contaminated habitats has been hampered by the fact that its natural habitat is not soil. Notwithstanding, the successes of *P. chrysosporium* in laboratory biodegradation studies ensure that intensive research on this species will continue. These successes have also stimulated quests for other filamentous fungi with the potential for degrading xenobiotics.

WOOD ROTS, LITTER ROTS, AND OTHER FILAMENTOUS FUNGI

There are more than 1,500 different species of white rot fungi. In addition, there are thousands of other fungal species loosely categorized as brown rots, dry rots, litter rots, soft rots, mycorrhiza, terricolous, and so forth. Most of these species have never been studied in the laboratory and represent a large potential for biodegradation research. In recent years, several groups have done comparative studies of white rot fungi with the expectation of finding better lignin-degrading systems (45, 46, 136). Others have taken advantage of this resource by screening isolates from culture collections and natural habitats against a variety of pollutants. Many new species with bioremediation potential have been identified (58, 118, 170). In a number of cases, these fungi show more practical promise than *P. chrysosporium* since their growth strategies offer better sustainability in natural habits; for example, they do not require constant additions of wood or other substrates (73). In addition, their enzymatic repertoires offer fresh approaches to xenobiotic degradation. In the survey by Gramss et al. (71), 58 species from different physioecol-

TABLE 2 Examples of xenobiotics degraded or transformed by *P. chrysosporium*

Type of compound	Examples	Reference(s)
Aromatic hydrocarbons	Benzo[*a*]pyrene	75
	Phenanthrene	27, 125, 157
	Pyrene	76
Chlorinated organics	Alkyl halide insecticides	103
	Atrazine	126
	Chloroanilines	11
	DDT	28, 110
	Pentachlorophenols	111, 122
	Trichlorophenol	99
	Polychlorinated biphenyls, Aroclor	44, 47
	Polychlorinated dibenzo-*p*-dioxins	70, 76, 164
	Trichlorophenoxyacetic acid	145
Nitrogen aromatics	2,4-Dinitrotoluene	162
	TNT	57
	Hexahydro-1,3,5-trinitro-1,3,5-triazine	56
Miscellaneous	Dyes	38, 130, 134, 135

ogical groups (wood degrading, wood and straw degrading, terricolous, ectomycorrhizal, and mitosporic) were grown in liquid cultures and tested against a battery of polyaromatic hydrocarbons. On average, wood-degrading species were best at metabolizing polyaromatic hydrocarbons, but competent fungi were found in all five groups. Polyaromatic hydrocarbon conversion was correlated with the production of MnP, peroxidase, and laccase (71).

Two particular white rot species that have received considerable attention are *Pleurotus ostreatus* and *Trametes versicolor*. They are both efficient at mineralizing polyaromatic hydrocarbons (20, 165) and at degrading polychlorinated biphenyls (174). *P. ostreatus* is better able to colonize soils than *P. chrysosporium* (129), and although it is a successful lignin-degrading species, it does not exhibit LiP activity (77). *P. ostreatus* does, however, produce several laccase isoenzymes encoded by families of laccase genes (64, 65). Laccase (*p*-diphenol-dioxygen oxidoreductase [EC 1.10.3.2]) is a member of the "blue copper oxidase" family (104, 159, 172). Laccases oxidize substrates by one-electron transfer steps and are active against lignin model compounds in the presence of mediators (93, 116). Many phenols and chlorophenols are transformed by these enzymes to radicals that subsequently undergo spontaneous polymerizations. Laccase-mediator combinations also show activity against acenaphthene, acenapthylene, and anthracene (94, 95). A purified laccase isolated from *T. versicolor* could oxidize a variety of polyaromatic hydrocarbons in vitro, including anthracene, benzo[*a*]pyrene, fluoranthene, and chrysene (115). In addition to laccase, an enzyme with properties of both LiP and MnP has been isolated from species of *Pleurotus* and *Bjerkandera*. This "third type" of lignin peroxidase shares catalytic properties of MnP (efficient oxidation of Mn^{2+} to Mn^{3+}) and LiP (oxidation of lignin model dimers) and has a high affinity for substituted hydroquinones (121, 147).

A representative list of filamentous fungi with the ability to degrade xenobiotics is presented in Table 3. Cell-free studies with enzymes from some of these species have been conducted. For example, a cell-free system of the MnP of the white rot fungus *Nematoloma frowardii* is capable of mineralizing pentachlorophenol, catechol, and pyrene. The ratio of MnP activity to the concentration of reduced glutathione affects the extent of mineralization (85).

Brown rot fungi degrade the cellulose and hemicellulose components of wood, leaving a residue of modified lignin that is dark brown. Although they are enormously destructive of wood products, the mechanism of wood decay by brown rot fungi has received far less attention than that of wood decay by white rot fungi (173). It is believed that the early steps in brown rot decay are nonenzymatic. Early studies demonstrate that the cellulose in wood can be depolymerized by Fenton's reagent [H_2O_2 plus Fe(II)], and most contemporary models invoke a Fenton-type extracellular system that produces hydroxyl radicals or other powerful oxidants that then attack the wood components (72). Different mechanisms proposed for hydroxyl radical production by brown rot fungi have been reviewed by Hyde and Wood (90). In studies on *Gloeophyllum trabeum*, 4,5-dimethoxycatechol and 2,5-dimethoxyhydroquinone have been implicated as electron transport carriers to Fenton's reactions (133). The ability of brown rots to degrade xenobiotics is a relatively new avenue of research. Current studies have implicated two species of *Gloeophyllum* in the degradation of chlorophenols (52, 149), and *G. trabeum* is active against polyethylene glycol (105) and ciprofloxacin, a fluoroquinolone antibiotic (167). No doubt many other interesting brown rot species will be found to demonstrate activity against a spectrum of xenobiotics.

MYCOREMEDIATION

Many workers divide bioremediation strategies into three general categories: (i) the target compound is used as a carbon source, (ii) the target compound is enzymatically attacked but is not used as a carbon source (cometabolism), and (iii) the target compound is not metabolized at all but is taken up and concentrated within the organism (bioaccumulation). Although fungi participate in all three strategies, they are often more proficient at cometabolism and bioaccumulation than at using xenobiotics as sole carbon sources.

The attributes that distinguish filamentous fungi from other life-forms determine why they are good biodegraders. First, the mycelial growth habit gives a competitive advantage over single cells such as bacteria and yeasts, especially with respect to the colonization of insoluble substrates. Fungi can rapidly ramify through substrates, literally digesting their way along by secreting a battery of extracellular degradative enzymes. Hyphal penetration provides a mechanical adjunct to the chemical breakdown effected by the secreted enzymes. The high surface-to-cell ratio characteristic of filaments maximizes both mechanical and enzymatic contact with the environment. Second, the extracellular nature of the degradative enzymes enables fungi to tolerate higher concentrations of toxic chemicals than would be possible if these compounds had to be brought into the cell. In addition, insoluble compounds that cannot cross a cell membrane are susceptible to attack. Fungi even solubilize low-rank coal, a particularly persistent, irregular, and complex polymeric substrate, although they do so slowly (33, 53, 132). Finally, since the relevant enzymes are usually induced by nutritional signals independent of the target compound during secondary metabolism, they can act independently of the concentration of the substrate, and their frequently nonspecific nature means that they can act on chemically diverse substrates.

Among filamentous fungi, *Phanerochaete chrysosporium* has emerged as the model system for studying xenobiotic degradation. A great deal remains to be learned about the fundamentals of how this white rot fungus mineralizes pollutants; not surprisingly, even less is known about the degradative mechanisms used by fungi in general. Oxidative enzymes play a major role, but organic acids and chelators excreted by the fungus also contribute to the process. Many of the toxic chemicals mineralized by fungi are already highly oxidized. The ability of fungi to lower the pH of their environment appears to be involved in the reduction of some of these compounds (17).

What about the future? Various brown rots, litter rots, aquatic fungi, anaerobic fungi, and mycorrhizal fungi, in conjunction with pollutant-tolerant plants, all provide opportunities for new research. Genetic engineering is another frontier. Fungal genes for degradative enzymes can be added to bacteria; alternatively, competent fungi can be modified to grow in an extended range of environments. For example, several groups have investigated the recombinant expression of fungal peroxidases in order to facilitate large-scale commercial production of these enzymes. LiP and MnP have been produced in an *Aspergillus niger* host and in the insect baculovirus expression system, although the LiP was not active (35, 96). Another development is

TABLE 3 Representative xenobiotic-degrading filamentous fungi

Group	Species	Substrate(s)	Reference(s)
White rot fungi	*Agrocybe aegarita*	Benz[*a*]anthracene	118
	Agrocybe praecox	Phenanthrene, pyrene	71
	Clitocybula duseni	Lignite	86
	Coriolopsis gallica	Anthracene, phenanthrene, pyrene	137
	Dichomitus squalens	Benz[*a*]anthracene	118
	Doedoela quercina	Benz[*a*]anthracene	118
	Ganoderma applanatum	Benz[*a*]anthracene	118
	Hypholoma fasciculare	Anthracene, fluoranthene, pyrene	71
	Kuehneromyces mutabilis	Anthracene, fluoranthene, phenanthrene, pyrene	71
	Lentinus edodes	Benz[*a*]anthracene	118
	Lenzites betulina	Anthracene, phenanthrene	71
	Nematoloma frowardii	Dinitrotoluene and trinitrotoluene, lignite coal, pentachlorophenol	85, 86, 148
	Pleurotus dryinus, P. eryngii, P. fusculatus, P. flabellatus, P. pulmonarius, P. sajor-caju	Benz[*a*]anthracene	118
	Pycnoporus cinnabarinus	Dibenzofuran	97, 118
	Stropharia rugosoannulata	Anthracene, fluoranthene, phenanthrene, pyrene	71, 118
	Trametes hirsuta	Textile dyes	1
Mycorrhizal fungi	*Morchella conica*	Anthracene, fluoranthene, phenanthrene	71
	Tylospora fibrilosa	Fluorobiphenyl	73
Others	*Agaricus bisporus*	Anthracene, fluoranthene, phenanthene, pyrene	71
	Coprinus comatus	Anthracene, fluoranthene, phenanthrene, pyrene	71
	Crinipellis stipitana	Pyrene	112
	Gloeophyllum striatum	Dichlorophenol	52
	G. trabeum	Pentachlorophenol	149
	Marasmiellus troyanus	Benzo[*a*]pyrene	171
	M. rotula	Pyrene	112

that of large-scale DNA sequencing. As this review is being completed, the genomic analysis of white rot fungi is being initiated. The U.S. Department of Energy is using a whole-shotgun approach to sequence the genome of *P. chrysosporium* (D. Cullen, personal communication). The availability of DNA sequence data for the model white rot fungus, coupled with the capacity to build DNA microarrays for transcriptional profiling and gene discovery, will provide powerful tools for identifying genes for hitherto undiscovered degradative enzymes from other filamentous fungi.

As we get better at recognizing what can and cannot be done with bioremediation, we will create a menu of choices using a broad range of organisms. In some situations, bioconcentration of a toxic waste is the best we can do. In others, the nonspecificity of the white rot fungi is ideally suited to treating low concentrations of mixed wastes in a nutrient-deficient habitat. In yet others, anaerobic bacteria are clearly the best candidates. Microbiologists know that pure cultures are rare in nature. Common sense tells us that in the real world, complete pathways of degradation are more likely to occur through the combined effects of many organisms. For example, cocultures of the bacterium *Stenotrophomonas maltophilia* and the fungus *Penicillium janthinellum* degrade high-molecular-weight polycyclic aromatic hydrocarbons more efficiently than does either microorganism alone (23).

Judicious combinations of chemical and physical processes with biological schemes also offer promise. Filamentous fungi, yeasts, and nonphotosynthetic bacteria are the workhorses of biological degradation. Therefore, it is ironic that the popular press has chosen the word "green" to describe environmentally friendly technologies such as bioremediation. Decidedly not green in color (except for a few spore types) and most certainly underappreciated (even by most microbiologists), the fungi possess the most varied and most efficient battery of depolymerizing enzymes of all decomposers. When joined with their bacterial brethren in cooperative catabolism, fungal-bacterial consortia will foster the ecological recovery of contaminated habitats worldwide. Filamentous fungi will always be major players in the "greening" of toxic waste sites and other polluted habitats.

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