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Fungal Decomposition of Natural Aromatic Structures and Xenobiotics: A Review

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Abstract—The review deals with transformation of natural and synthetic aromatic compounds by fungi (causative agents of white rot, brown rot, and soft rot, as well as soil filamentous fungi). Major enzyme types involved in the transformation of lignin and aromatic xenobiotics are discussed, with emphasis on activity regulation under the conditions of secondary metabolism and oxidative stress. Coupling of systems degrading polysaccharides and lignin and non-phenolic lignin structures (without the involvement of lignin peroxidase) is analyzed, together with nonenzymatic mechanisms involving lipoperoxide free radicals, cation radicals, quinoid mediators, or transition metal ions. Metabolic pathways resulting in the formation of aromatic and haloaromatic compounds in fungi are described. Consideration is given to the mechanisms of fungal adaptation to aromatic xenobiotics.

The last several decades have been characterized by a steady increase in biospheric pollution by diverse aromatic xenobiotics (haloaromatic pesticides, polycyclic aromatic compounds, chlorinated biphenyls, dioxins, etc.). Microorganisms capable of detoxifying these pollutants under natural conditions are of particular interest in this connection. In recent years, preferential attention has focused on the role of soil bacteria in degradation of stable aromatic xenobiotics. Fungi also have significant potential for degradation of aromatic compounds, although the underlying mechanisms are not necessarily transparent in every case. It is generally held that such degrader activity of fungi is based on their ability to metabolize vegetable substrates. This applies primarily to basidiomycetes, causative agents of white rot, which are the most efficient degraders of lignin (a reticular phenylpropanoid polymer of irregular structure) [1–6]. However, the ability to degrade xenobiotics is not limited to basidiomycetes; it is also found in other xylotrophs (e.g., those causing brown rot and soft rot), as well as in a broad range of soil mycelial fungi [1].

This review provides a state-of-the-art analysis of the problem of fungal transformation of aromatic compounds.

Abbreviations: AAO, aryl alcohol oxidase; ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate); ADD, aryl aldehyde dehydrogenase; ALD, aryl alcohol dehydrogenase; VA, veratryl alcohol; DMB, dimethoxybenzene; DMBQ, dimethoxybenzoquinone; DMH, dimethoxyhydroquinone; CDMB, 2-chloro-1,4-dimethoxybenzene; DCDMB, 2,6-dichloro-1,4-dimethoxybenzene; LP, lignin peroxidase; Mn-P, manganese peroxidase; HAA, 3-hydroxyanthranylic acid; SOD, superoxide dismutase; TCHQ, tetrachloro-1,4-hydroquinone; CDH, cellobiose dehydrogenase; CA, cinnabarinic acid; PAL, phenylalanine ammonia lyase.

XYLOTROPHS: CAUSATIVE AGENTS OF WHITE ROT

Extracellular enzymes. Lignin is degraded by fungi of diverse taxonomic groups, ascomycetes in particular [1–3], but basidiomycetes are the most efficient in this respect.

These fungi produce several types of redox enzymes, described below.

Heme-containing peroxidases: lignin peroxidases (LPs; EC 1.11.1.14), manganese peroxidases (Mn-Ps; EC 1.11.1.13), and peroxidases with a broad spectrum of specificities (EC 1.11.1.7). Flavine oxidases: glucose oxidases (EC 1.1.3.4); pyranose-2-oxidases (EC 1.1.3.10); methanol oxidases (EC 1.1.3.13); and aryl alcohol oxidases (AAOs; EC 1.1.3.7), which reduce dissolved oxygen to peroxide and, at the same time, oxidize hydroxyls of appropriate substrates to carbonyls. Cellobiose dehydrogenases (CDHs; EC 1.1.99.18): flavohemoproteins that reduce phenoxy radicals and cation-radicals, ferric ions, or quinones and, at the same time, oxidize cellobiose to cellobionolactone. Copper-containing oxidases: laccases (EC 1.10.3.2), which reduce dissolved oxygen to water and, at the same time, oxidize phenolic and non-phenolic substrates with the formation of quinones or phenoxy radicals and cation radicals, and glyoxal oxidase and galactose oxidase (EC 1.1.3.9), which reduce oxygen to hydrogen peroxide and, at the same time, oxidize an appropriate alcohol or aldehyde group [1, 4].

In addition to lignin-degrading enzymes, these fungi produce systems of hydrolases cleaving pectin, hemicelluloses, and cellulose; such hydrolytic systems comprise two types of CDHs (EC 3.2.1.91) and a set of endoglucanases (EC 3.2.1.4), xylanases (EC 3.2.1.8, 3.2.1.37, and 3.2.1.72), mannanases (EC 3.2.1.78 and

3.2.1.100), glycosidases (EC 3.2.1.20–3.2.1.25, 3.2.1.40, 3.2.1.51, and 3.2.1.55), acetylxylanases, and feruloylesterases, which ensure consequential and complete degradation of the polysaccharide moiety of wood [5].

Naturally occurring strains of white rot fungi are characterized by coupled degradation of all wood components, but this process does not necessarily require the presence of CDH and cellulases. Laccase is a mandatory enzyme, since laccase-deficient mutants completely lose the ability to degrade lignin [6]. Polysaccharides do not constitute a barrier to ligninolysis.

Lignin-degrading enzymes are believed to act at the surface of hyphae contacting the cell wall. However, degradation is not limited to contact areas; it also occurs over the whole internal surface of the lumen (if one or two hyphae are present there) and even in the depth of the secondary wall.

Lignin is not metabolized completely, and some part of it undergoes conversion into condensed products. In model experiments, laccase-catalyzed ligninolysis resulted in the formation of biphenyl structures. The condensing activity of laccase was also demonstrated in a culture of oyster mushrooms grown on lignosulfonate [1].

In addition, there are nonenzymatic mechanisms of ligninolysis, involving (a) the system ferrous ions–hydrogen peroxide (the so-called Fenton reagent); (b) complexes of transition metal ions; (c) peroxide, lipoperoxide, and superoxide radicals; and (d) endogenous redox mediators transporting redox equivalents between the hypha and the matrix of lignin.

Thus, ligninolytic fungi are equipped with enzymes effecting thorough transformation of aromatic structures. Of major importance are LPs, Mn-Ps, and laccases, capable of effecting both direct and mediator-aided oxidation of non-phenolic structures. However, the changes in the structure of the substrate are not limited to the effects of these enzymes.

Regulation of activity of lignin-degrading enzymes under the conditions of secondary metabolism. During the stage of secondary metabolism, including in the absence of the natural substrate, many xylotrophs synthesize odoriferous aromatic compounds [7–11]. The diversity and total amount of such aromatic metabolites depend on the type of the fungus and the spectrum of enzymes it produces. The compounds in question are formed in fungi that cause both brown (destructive) rot and white (corrosive) rot. Only in white rot fungi, however, did the need for secondary metabolism of endogenous aromatic compounds lay the groundwork for an adaptive mechanism whereby the trophic base was extended through lignin utilization.

White rot fungi synthesize lignin-degrading enzymes when grown on media not necessarily requiring natural lignin-containing substrates. In the absence of a cosubstrate, fungi are incapable of using lignin as a sole source of carbon and energy. As a rule, the formation of lignin-degrading enzymes does not coincide with the synthesis of polysaccharide hydrolases (the activity of the latter attains its maximum during trophophase).

The formation of multiple forms of LP, Mn-P, and laccase is a common characteristic of ligninolytic fungi, such as *Phanerochaete chrysosporium*. The change in the spectrum of these enzymes and the decrease in the activity of LP observed at the stage of idiophase in *P. chrysosporium* are partly due to proteolytic degradation [12]. Both extra- and intracellular proteases are involved in the regulation of laccases and nonspecific peroxidases in *Trametes versicolor* during the switch from primary growth (trophophase) to secondary growth (idiophase) under the conditions of nitrogen or carbon starvation [13].

During static culturing of *P. chrysosporium* in a medium with glucose, the attainment of maximum lignin degrader activity falls behind biomass accumulation (the completion of which coincides with exhaustion of the source of nitrogen). This maximum is observed with the onset of idiophase, which is characterized by the formation of veratryl alcohol (VA). Veratrole may be of key importance for the synthesis and function of LP (as its endogenous inducer, stabilizer, and mediator). Still, this compound is a typical secondary metabolite of the fungus, which is not involved directly in ligninolysis. Thus, processes of secondary fungal metabolism, associated with deamination of aromatic amino acids under nitrogen deficiency, may result in the formation of lignin-degrading enzymes (and of LP first of all).

Aryl alcohol oxidase (AAO) is yet another enzyme (in addition to LP) involved in the formation of hydroxylated and methoxylated aromatic compounds in *Bjerkandera adusta* and *Pleurotus eryngii* [14, 15]. This enzyme supplies the hydrogen peroxide required for LP operation [15]. *P. eryngii* possesses an intracellular aryl aldehyde dehydrogenase (ADD) and an aryl alcohol dehydrogenase (ALD) [16]. An intracellular ALD has also been isolated from *P. chrysosporium* [17]. Intracellular metabolism of natural precursors of veratrole accounts for both the intracellular location of LP in many fungi and its release at the stage of mycelium autolysis. It may be that LP was formed in evolution as a means of detoxification of intracellular deamination products of aromatic amino acids, e.g., under the conditions of oxygen starvation (Fig. 1), and that the extracellular function of lignin decomposition was acquired subsequently.

The ratio of non-hydroxylated, hydroxylated, and methoxylated benzoate derivatives may vary in fungi of different species. Very possibly, it depends on the degree of toxicity of certain aromatic metabolites to a given fungal species. Thus, in the case of *Ceriporiopsis subvermispora* and *Cyathus stercoreus*, the most toxic aromatic compounds are benzaldehyde derivatives. At concentrations below 1 mM, aromatic compounds stimulated the growth of hyphae; within the range 5–10 mM, the growth was either retarded or

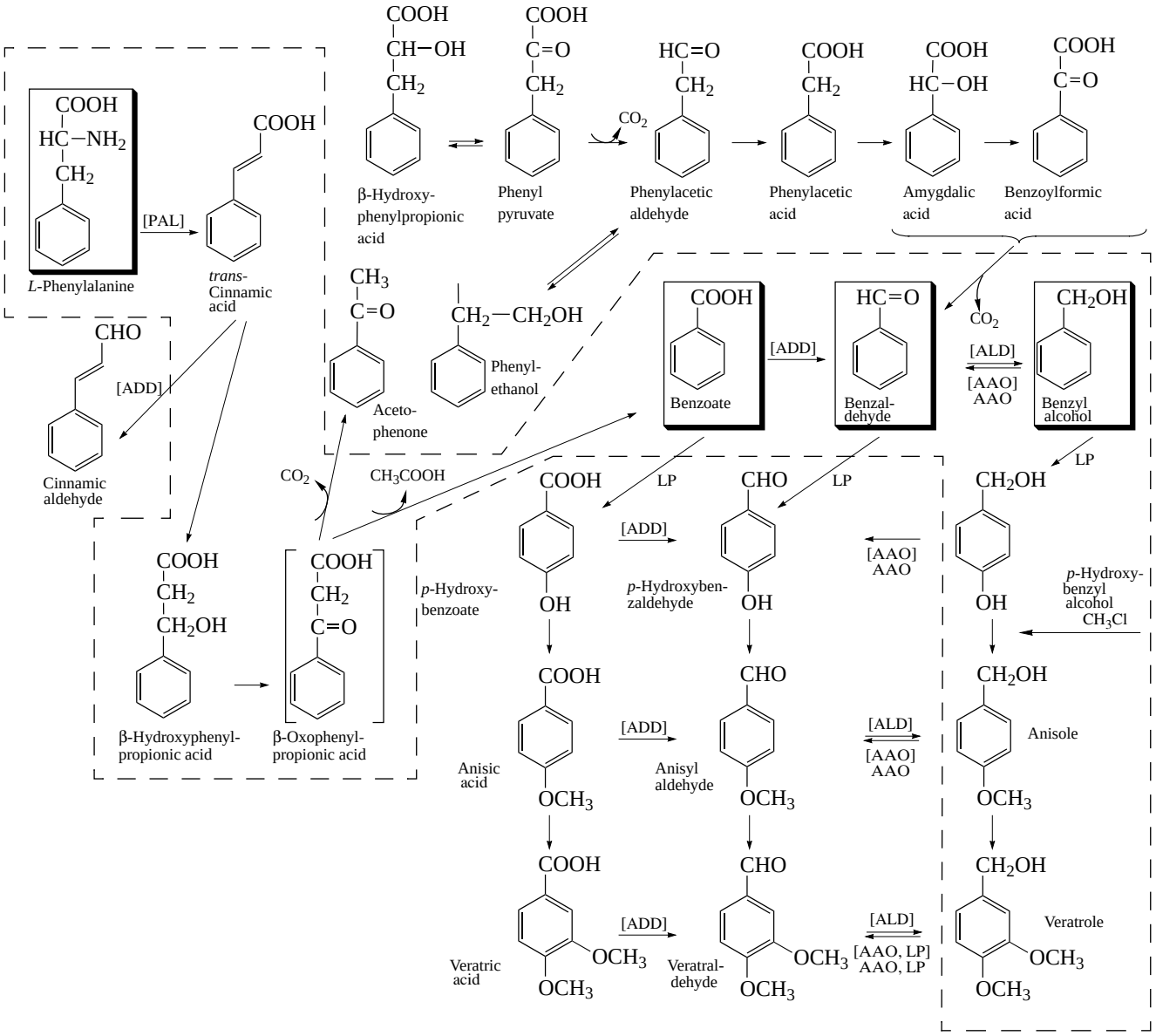

Fig. 1. Structural formulae of secondary metabolites of *L*-phenylalanine in *B. adusta*. The dashed line separates the schematic representation of phenylalanine metabolism in *P. chrysosporium*, which results in veratrole formation. Intermediates in brackets are putative; enzymes in brackets are intracellular.

stopped. Detoxification of exogenous derivatives of benzoic acid, cinnamic acid, and benzaldehyde introduced into the culture of white rot fungi required the involvement of lignin-degrading enzymes. Monomethoxylated compounds are more toxic than derivatives carrying two or more methoxy functions. Of the two fungal species in question, *C. stercoreus* (which produces more Mn-P) is considerably more sensitive to such compounds than *C. subvermispora* (which produces more laccase). Di- and trimethoxylated compounds are more potent enzyme inducers than their hydroxylated counterparts [18].

There is evidence suggesting that the mechanisms of veratrole formation in fungi are not limited to phenylalanine deamination; alternative sources of this compound include various phenylpropanoid compounds and non-phenolic lignin structures. This may account for the stimulation of lignin degrader activity observed in fungi in the presence of lignin, its derivatives, and certain model compounds [19].

Lignin is not an essential inducer of lignin-degrading enzymes. Products of fungal wood decay, such as *p*-hydroxycinnamic and ferulic acids, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoate, vanillin, and vanillic acid [20], may serve as precursors of veratrole and other endogenous aromatic inducers stimulating the synthesis of lignin-degrading enzymes.

The formation of haloaromatic (in addition to aromatic) metabolites is a characteristic feature of ligninolytic fungi. Chlorinated 1,4-dimethoxybenzenes are formed by diverse fungal species. *Bjerkandera* sp. BOS55 produces not only veratrole, veratraldehyde, and anisyl aldehyde, but also 3-chloro-anisyl aldehyde and an unidentified aromatic compound carrying two chlorine atoms. The latter metabolite has also been identified in *B. adusta*, but not in *P. chrysosporium* [21].

Other chlorinated aromatic compounds found among endogenous fungal metabolites include 2-chloro-1,4-dimethoxybenzene (CDMB), 2,6-dichloro-1,4-dimethoxybenzene (DCDMB), tetrachloro-1,4-dimethoxybenzene (also known as drosophilin A methyl ester), and tetrachloro-4-methoxyphenol (drosophilin A) [22–25]. CDMB is a derivative of 1,4-dimethoxybenzene (DMB), formed de novo by smoky bracket (*B. adusta*) and wood blewit (*Lepista nuda*) [26]. In the metabolism of aromatic compounds, CDMB serves as a mediator of LP-catalyzed anisole oxidation to anise aldehyde [27, 28].

Effects of oxidative stress. Stimulation of ligninases by nitrogen deficiency is not a general rule. In *Pleurotus ostreatus* and *Lentinus edodes*, this activity is found in a medium with high nitrogen content. In *Coriolopsis gallica* and *Bjerkandera adusta*, nitrogen-rich bran-containing medium stimulates the formation of LP, Mn-P, veratryl alcohol oxidase, and laccase to a greater extent than the inducer of laccase activity 2,5-xylidine or a mixture of LP and Mn-P inducers (VA plus Mn ions) [29]. Secondary metabolism and synthesis of ligninases in *Phanerochaete chrysosporium* may be induced by limiting the sources of carbon and sulfur. Substitution of pure oxygen for air increases two- to threefold ligninase activity of a static culture of *P. chrysosporium*, grown without shaking.

The majority of isoforms of LP, Mn-P, and laccase are individual proteins encoded by distinct structural genes. The number of isoforms and their types strongly depend on the conditions of *P. chrysosporium* culturing. Increasing the concentration of Mn^{2+} in the medium switches the synthetic activity of fungal cultures (*P. chrysosporium*, *P. flavido alba*, *P. magnoliae*, *Phlebia radiata*, *Ph. tremellosa*, *Ph. subseralis*, *Lentinus edodes*, and *Phellinus pini*) from LP to Mn-P [30].

When grown under the conditions of nitrogen limitation, *Phanerochaete laevis* (a species closely related to *P. chrysosporium*) exhibits a high activity of Mn-P, which is regulated by the level of Mn^{2+} in the medium. Laccase activity is also detected in such media; whatever the levels of Mn^{2+}, however, attempts to register LP activity do not meet with success [31].

In certain fungi (e.g., *P. tigrinus*, *P. eryngii*, and *Bjerkandera* spp.), a single enzyme combines the properties of LP and Mn-P [6, 32, 33]. Accordingly, its synthesis is not suppressed by excess Mn^{2+}.

Conflicting evidence regarding the effects on the activity of fungi of (a) carbon and nitrogen sources and (b) Mn^{2+} and oxygen concentrations in the culture medium may be accounted for by the effects of these factors on each other. The formation of ligninolytic oxidoreductases is viewed as a response of the fungi to oxidative stress induced by conditions of culturing.

Static culturing on glucose under the conditions of excess oxygen and nitrogen or carbon limitation was considered for a long time as the only acceptable method favoring the formation of LP in *P. chrysosporium* [34, 35]. This view was questioned when the fungus grown on media with cellulose replacing glucose exhibited high activities of LP [36]. The culture grown on glucose is believed to synthesize an extracellular polysaccharide that takes up excess sugar from the medium. The polysaccharide prevents LP induction by restricting oxygen diffusion to the hyphae. These effects do not take place when the fungus is grown on cellulose.

When grown in oxygenated medium under the conditions of glucose limitation (these two factors are necessary for LP induction), *P. chrysosporium* loses the cellular organization of its hyphae due to toxic effects of excess oxygen. A similar loss of the ultrastructural organization of the cells is noted when LP is formed in cultures grown on cellulose-containing media under shaking and normal aeration. In this case, the hyperoxidated state of the cells may be caused by the rate of carbon supply from cellulose, which is insufficient for maintaining oxygen homeostasis. These findings suggest that LP synthesis may be a response to oxidative stress [37].

This understanding is supported by the observation that mitochondria of *P. chrysosporium* cells lose succinate dehydrogenase and cytochrome oxidase activities when grown on glucose. In an atmosphere of pure oxygen, the cells form LPs, which protect them from accumulation of reactive oxygen species. Cells grown on cellulose retain the normal function of mitochondria and synthesize LPs without additional oxygenation. It is believed that, in cells grown on cellulose, the function of mitochondria may be limited by the glucose supply, which in turn results in accumulation of reactive oxygen species even in the absence of additional oxygenation [38].

High Mn^{2+} concentrations and oxygen deficiency are known to decrease the rate of mineralization of synthetic lignin by the fungus *P. chrysosporium* [39]. Mn^{2+} is capable of acting as an antioxidant and a scavenger of free radicals generated in the Fenton reaction ($Fe^{2+} + H_2O_2$) [40–42]. In addition, Mn^{2+} is a cofactor of Mn-dependent SOD (Mn-SOD); by virtue of its ability to convert superoxide radicals into the less dangerous peroxide, this enzyme serves as a major factor of cell defense against oxidative stress. Thus, the regulatory role of Mn^{2+} in the formation of LPs may be accounted for by the effect of Mn^{2+} on the level of oxygen radicals in the fungus. The high level of oxygenation required for LP formation in *P. chrysosporium* regardless of the

content of nitrogen in the medium may be attained by decreasing the level of Mn^{2+} in this medium. Under such conditions, the activity of Mn-SOD is also inhibited [43].

Mester *et al.* [44] demonstrated that, in *Bjerkandera* spp. and *P. chrysosporium*, Mn^{2+} deficiency stimulates endogenous formation of VA and increases the content of LP. The absence of Mn-SOD activity under the conditions of Mn^{2+} deficiency and increasing concentration of reactive oxygen species generated in the cells in response to oxygenation requires that the formation of alternative antioxidant enzymes be stimulated simultaneously. The observation that the level of LP increases when other antioxidative enzymes (catalase and SOD) are suppressed [45] suggested an antioxidant function for LP.

Thus, the dependence of the synthesis of LP and veratrole in *P. chrysosporium* on the level of glucose, oxygen, and nitrogen consumption may be accounted for by the ability of these factors to induce oxidative stress. In the absence of Mn^{2+} and Mn-SOD, excess superoxide radicals and peroxide formed in the culture of the fungus stimulate the synthesis of LP, which efficiently eliminates peroxide. When *P. chrysosporium* is grown under nitrogen limitation, the activity of catalase is also increased (by 5–10 times) [46].

Oxidative stress appears to regulate the transcription of the gene encoding Mn-SOD [47]. However, the mechanism of regulation is more complicated than in the case of LP, because the expression of *mnp* genes in diverse white rot fungi requires Mn^{2+}, which is an antioxidant and an inducer of Mn-SOD at the same time [49].

Convincing evidence of regulation of other ligninolytic enzymes by oxidative stress is not available. Copper cations are known to stimulate fungal synthesis of laccases, as well as the synthesis of Zn/Cu-SOD (the situation is similar to that of Mn-P and Mn-SOD, the synthesis of which is regulated by Mn^{2+}). *P. chrysosporium* synthesizes laccase when grown on nitrogen-rich medium with cellulose (but not glucose) as the source of carbon [50], and this is also similar to the case of LP synthesis. Conversely, the synthesis of laccase attains high levels in *Ganoderma lucidum* and *Irpex lacteus* grown on nitrogen-rich medium in the presence of glucose [51]. In *G. lucidum*, syringic acid and leaf lignin (containing syringyl residues) are supplementary stimulants of laccase production [52, 53].

The rate of mineralization of synthetic lignin in the fungus *Cyathis stercoreus* is maximum in the presence of 1 mM ammonium tartrate and 1% glucose, although the activities of extracellular enzymes are low. In the presence of 10 mM ammonium tartrate and 1% glucose, the rate of mineralization in threefold lower, whereas the levels of laccase and Mn-P are high. A static, aerated culture forms more enzymes than its counterpart grown under shaking [54].

As a rule, growth of ligninolytic fungi on nitrogen-rich media also stimulates the synthesis of peroxidases with a broad spectrum of specificities; an example of such fungi is *Phellinus iginarius* [55].

Coupling of polysaccharide- and lignin-degrading systems may be achieved via three metabolic pathways: (1) carbohydrate catabolism (which ensures the viability of fungi at the stage of secondary metabolism [56]); (2) generation of H_2O_2 for LP and Mn-P by sugar oxidases; and (3) low-molecular-weight mediator recovery in the coupled system laccase (peroxidase)–cellobiose: quinone oxidoreductase (CDH).

Soluble carbohydrates are formed by cellulase/hemicellulase systems of xylotrophic fungi. In *Phanerochaete chrysosporium*, the enzymes of this group are very similar to those of the polysaccharide hydrolase system of the white rot fungus *Trichoderma reesei* [57]. Glucose oxidase and pyranose-2-oxidase, synthesized by lignin degraders grown on glucose, exemplify extracellular oxidoreductases that are coupled to a cellulase–hemicellulase system and capable of forming H_2O_2 [58]. In addition to these two extracellular enzymes, *P. chrysosporium* possesses mycelium-associated systems generating H_2O_2 when the fungus is grown on glucose under nitrogen deficiency [46].

Of particular interest among extracellular enzymes involved in coupling cellulose and lignin catabolism is CDH. This oxidase, found in all xylotrophs, is specific for reducing sugars containing a 1,4-β-glycoside bond (cellobiose and cellooligosaccharides, mannobiose and mannooligosaccharides, and lactose), but not monosaccharides or their oligomers with an α-glycoside bond [59, 60]. The enzyme oxidizes the reducing residue of a di- or oligosaccharide to the corresponding lactone while oxidizing a two-electron or single-electron acceptor. Quinones, phenoxy radicals, and cation radicals (formed by laccases or peroxidases) or ions (Fe^{3+}, Cu^{2+}, and Mn^{3+}) are all capable of acting as such electron acceptors. Oxygen reduction to H_2O_2, characteristic of other sugar oxidases, is not typical for CDH (even though it may still take place).

Synthesis of CDH in *P. chrysosporium* is observed when the fungus is grown on cellulose or wood. Synergistic effects of CDH and Mn-P are due to the formation of Mn^{3+} [61]. CDH is not a unique enzyme of *P. chrysosporium* (*Sporotrichum pulverulentum*); it has also been found in *Heterobasidion annosum* [62], *Schizophyllum commune* [63], *T. versicolor* [64], and *Pycnoporus cinnabarinus* [65].

Pycnoporus cinnabarinus efficiently degrades lignin; of note, laccase is the only extracellular oxidase of this fungus. Laccase is also involved in the formation of the characteristic orange-red phenoxazinone pigment, cinnabarinic acid (CA). Its precursor is 3-hydroxyanthranylic acid (HAA), a tryptophan metabolite, which is believed to act as a mediator in laccase-catalyzed oxygenation of stable non-phenolic structures of lignin [66]. It is generally held that CDH controls the ratio of HAA and CA by reducing HAA oxidation intermediates (and thereby preventing their conversion to CA).

CDH is responsible for suppression of CA formation in *P. cinnabarinus* cultures grown on cellobiose or cellulose.

HAA oxidation products are natural electron acceptors in CDH-catalyzed reactions that take place in *P. cinnabarinus*. In the absence of cellulose, laccase oxidizes HAA to the end product, CA. In the presence of cellulose and/or cellodextrins, CDH reduces HAA intermediates, thereby preventing CA accumulation and maintaining the mediator at the level necessary for ligninolysis. The cycle operates, and ligninolysis remains coupled to cellulose degradation, while cellulose or cellobiose is present in the system. Evidence in support of this metabolic model comes from the observation that wood populated by *P. cinnabarinus* appears as affected by a typical white rot. CA and other structurally related phenoxazinone pigments are not accumulated until the onset of formation of fruiting bodies. It is not clear, however, which HAA intermediate is involved as a mediator in the degradation of non-phenolic lignin structures [65–68] (Fig. 2).

Degradation of non-phenolic lignin structures. Various mechanisms have been proposed for explanation of oxidation of non-phenolic lignin structures by fungi that involve Mn-P (or the hybrid LP/Mn-P) and laccase [6]. LP is capable of oxidizing non-phenolic structures of lignin directly; the cation radical intermediate formed in the reaction cleaves Cα–Cβ bonds. The ability to synthesize this enzyme is far from being a common feature of all white rot fungi [4, 6]. Moreover, recent data suggest that, for the majority of ligninolytic fungi, the presence of an LP (such as the enzyme typical for *P. chrysosporium*) is an exception, rather than the rule [6].

In addition to these enzymes, of importance to the process of degradation of non-phenolic lignin structures are low-molecular-weight mediators, capable of diffusion into the depth of the secondary wall, where they start generating free radicals. Interaction of these radicals with non-phenolic lignin structures facilitates their cleavage. The process may occur at a considerable distance from the hyphae, including locations beyond the reach of the enzyme molecules [69]. Such low-molecular-weight agents include lipoperoxides, sulfhydryl agents, transition metal ions, reactive oxygen species, and natural redox mediators with appropriate redox potential values, which are capable of transferring oxidizing equivalents from the active center of the enzyme to non-phenolic lignin structures [70].

Mn-P, which generates Mn^{3+}, a powerful oxidizing agent, may play a key role at the first stage of ligninolysis by a variety of fungi. Mn^{3+} oxidizes certain non-phenolic aromatic structures of lignin [71–76], as well as oxalate (a metabolic product of other fungi), resulting in the formation of free radicals that reduce Fe^{3+} and initiate reactions with the involvement of hydroxyl radicals [77].

Mn-P is the major enzyme degrading wheat straw in the basidiomycete *Nematoloma frowardii*. The fungus mineralizes up to 75% of synthetic polymeric lignin (8% is the corresponding value obtained in a cell-free system containing Mn-P, Mn^{2+}, H_2O_2, malate, and fumarate) [78]. *P. chrysosporium*, *Rigidoporus lignosus*, and *Ceriporiopsis subvermispora* produce multiple forms of Mn-P when grown on wood or sawdust. These isoforms, in addition to laccase, are major enzymes in *T. versicolor*, *Pleurotus* spp., and *Coriolopsis polyzona* grown on wheat straw, particularly during the early stage of cultivation [79–81].

In *Phlebia radiata*, the oxidation of linoleic acid (the main polyunsaturated fatty acid component of lipids in xylotrophic fungi) by Mn-P involves an H_2O_2-activated lipoperoxide mechanism. Lipids also stimulate mineralization of ^{14}C-labeled synthetic lignin and ^{14}C-labeled wheat straw effected by Mn-P [82]. Mn^{3+} functions as a generator of lipid free radicals and hydroperoxides in the course of lignin degradation [83–85]. Diffusion of lipoperoxide free radicals into cell wall areas that are not accessible to fungal hyphae makes it possible to oxidize non-phenolic lignin structures located there.

A mechanism of free radical generation not involving Mn-P [86] is believed to occur at the stage of idiophase as a result of autolysis of mycelium [87]. Lipoxygenases released during this process catalyze the formation of lipoperoxide free radicals from fatty acid substrates of membrane phospholipids.

Laccases of ligninolytic fungi are also capable of generating highly active free radicals. The functions of laccase may not be limited to oxidation and degradation of lignin structures with free phenolic groups [88]. The mechanism of hydroxyl radical generation in the fungus *Pleurotus eryngii* is based on the formation of semiquinone intermediates during the oxidation of lignin-derived hydroquinones by laccase [89]. Semiquinones cause nonenzymatic reduction of Fe^{3+} and molecular oxygen, resulting in the formation of Fe^{2+} and superoxide, respectively. Decomposition of superoxide radicals makes H_2O_2 available. The resulting hydroxyl radicals convert non-phenolic lignin structures into their phenolic counterparts, which can be further cleaved by laccase.

The role of laccase in the oxidation of non-phenolic lignin structures was studied by Leontievsky and colleagues [6, 90]. The data reported by these authors indicate that certain lignin degraders contain low-molecular-weight mediators extending the functions of laccase to cover activities that are conventionally ascribed to LP (or the system Mn-P/Mn^{2+} when it operates in the presence of agents generating free radicals).

Submerged cultivation of *Panus tigrinus*, *Coriolus versicolor*, *Phlebia radiata*, or *P. tremellosa* on wheat straw is associated with the formation of laccases that have a characteristic blue color and specific ESR spectra when purified (the so-called *blue laccases*).

Attempts to isolate blue laccases from solid-phase cultures of *P. tigrinus*, *Ph. radiata*, and *Agaricus bisporus*, grown on the same substrate, failed. The enzymes isolated in the latter case were yellowish brown in color (the so-called *yellow laccases*), and their ESR spectra differed from those of the blue laccases (of note, the *N*-terminal sequence of each such enzyme was identical to that of the blue counterpart isolated from the same fungus). Blue laccases oxidized veratrole and model nonphenolic lignin only in the presence of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonate (ABTS), which acted as a cation radical mediator, whereas yellow laccases did not require any exogenous mediation. Thus, solid-phase culturing of the laccase-type ligninolytic fungi results in modification of blue laccases by lignin degradation products acting as efficient endogenous redox mediators [91]. An important advantage of using laccase-based oxidation systems containing low-molecular-weight mediators (over LP or Mn-P) is that it eliminates the need for the presence of peroxide in the system [92].

BASIDIOMYCETES CAUSING BROWN (DESTRUCTIVE) ROT

These fungi selectively degrade the polysaccharide moiety of wood without affecting lignin significantly. They are the major participants in the natural turnover of biomass, and their contribution to soil remediation in coniferous woods is of prime importance [93]. On the other hand, brown rot fungi affect wooden buildings and stocked wood (deal in particular). Processes associated with brown rot development are typical for humification. The decayed wood contains high amounts of humic and himatomelanic acids (in the case of white rot, wood decay is associated with preferential production of fulvic acids) [20].

Extracellular enzymes. Intracellular laccase plays a significant role in humification processes associated with wood decay caused by brown rot. The release of the enzyme in the course of autolysis of aged hyphae accounts for the slight decrease (of no more than 10%) in the total content of lignin (the fungi involved are usually devoid of laccase and peroxidase activities) [94]. Nucleotide sequences homologous to laccase genes of white rot fungi are present in the genomes of brown rot fungi [52]. Certain fungal species causing brown rot (*Poria* spp. and *Tyromyces* spp.) may have the capacity for secondary metabolism of phenylalanine deamination products, which results in the formation of aromatic compounds (such as anisole and benzaldehyde) [7]. However, the enzymes of secondary metabolism in these fungi are not appropriate to ligninolysis.

Extracellular systems of brown rot fungi cannot degrade ordered cellulose or oxidize phenolic compounds. Their cultures contain cellobiohydrolase I. It is possible that this enzyme is induced by lignified substrates; an alternative explanation suggests that degradation of ordered cellulose is effected by hydrolase, rather than oxidase, systems [4]. The ability to hydrolyze soluble carboxymethylcellulose is documented for extracellular enzymes of (a) brown rot fungi grown on cellulose [95] and (b) certain other species, including *Lenzites (Gloeophillum) sepiaria* [96, 97]. Xylanases, mannanases, and mannosidases were isolated from the brown rot fungus *Tyromyces palustris* [1].

Brown rot fungi of the family Coniophoraceae form CDH [98, 99]; the formation of this enzyme by brown rot fungi of other families grown on natural substrates has been questioned [100].

Among other oxidoreductases, it is worth mentioning alcohol oxidase of *Poria contigua* [101], which differs from alcohol oxidases of white rot fungi, e.g., *Polyporus obtusus*. This enzyme may be capable of forming H_2O_2, which is needed for oxidative degradation; the extent to which such enzymes are widespread in brown rot fungi remains to be determined.

A new glyoxalate dehydrogenase has been isolated from a cell-free extract of *Tyromyces palustris*; this enzyme catalyzes dehydration of glyoxalate to oxalate in the presence of cytochrome *c*. Being a flavohemoprotein (as CDH), glyoxylate dehydrogenase uses FAD as a flavine cofactor (not FMN). The enzyme is of interest because oxalate may be involved in cellulose degradation reactions caused by brown rot fungi.

Nonenzymatic mechanisms used by brown rot fungi. In 1974, Koenigs [102] hypothesized that, unlike soft rot and white rot fungi, causative agents of destructive rot may use an alternative mechanism, not involving enzymes, which is based on free-radical reactions initiated by hydroxyl radicals. The latter are formed in the course of H_2O_2 reduction by Fe^{2+}:

$$Fe^{2+} + H_2O_2 \dashrightarrow Fe^{3+} + HO^{\bullet} + HO^{-}.$$

Destructive rot fungi are believed to cause wood decay primarily by this mechanism [58, 76, 99, 103–105].

The proposed mechanism of formation of hydroxyl radicals in the brown rot fungus *Coniophora puteana* [99] involves the following stages: (a) Fe^{3+} reduction by CDH [98] in the vicinity of the hyphae or at the cellular surface, (b) diffusion of Fe^{2+} thus formed into the depth of the wall of the plant cell, (c) formation of the complex Fe^{2+}–oxalate, and (d) formation of hydroxyl radicals at a safe distance from the fungal hyphae.

CDH, which has a high affinity for cellulose, may itself act at a distance from the hyphae, i.e., in the depth of cellulose lamellae, where its major substrate, cellobiose, is formed. Thus, Fe^{2+} recovery in the Fenton reaction, involving cellobiose (formed by cellulases) and CDH, may also take place in this species of xylotrophs.

The ability to synthesize CDH during growth on natural substrates has not been demonstrated for the majority of brown rot fungi [100]. Most likely, the presence of Fe^{2+} and dissolved oxygen alone is sufficient for

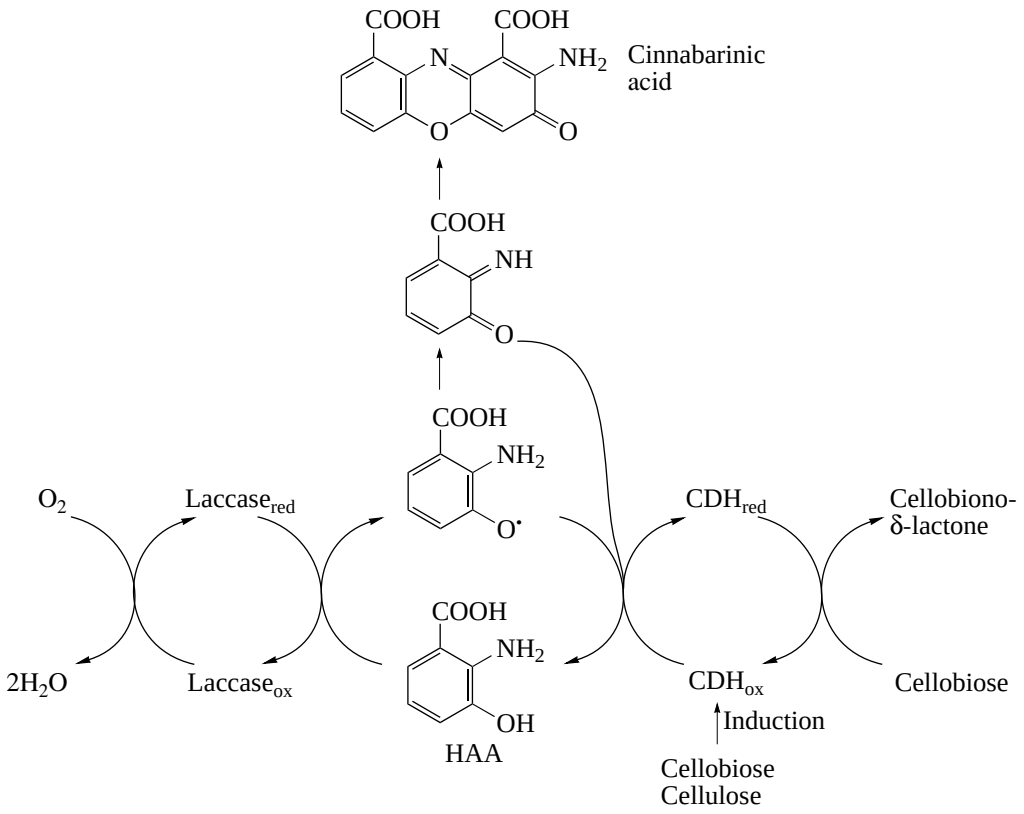

Fig. 2. Schematic representation of the putative mechanism of cyclic transformation of HAA in the presence of laccase and CDH (after [65, 67, 68]). Each consecutive intermediate in HAA oxidation depicted in the figure is formed via elimination of one hydrogen from the immediate precursor [9].

generating hydroxyl radicals [106]. H_2O_2 may be formed via Fe^{2+} autoxidation [99]:

$$2Fe^{2+} + O_2 + 2H_2O = 2Fe^{3+} + 2H_2O_2.$$

Nonenzymatic generation of hydroxyl radicals may also occur in the absence of iron ions. One of the mechanisms involves quinines, which replace metal ions [107, 108] (Fig. 3).

The brown rot fungus *Gloeophyllum trabeum* was shown to excrete 4,5-dimethoxy-1,2-catechol and 2,5-dimethoxy-1,4-benzoquinone when grown in the dark on a mineral medium supplemented with glucose [109]. These fungal compounds may serve as mediators in the Fenton reaction. Other aromatic metabolites formed by the fungus under such conditions include benzoate, ethylbenzene, and methyl phenylacetate.

SOIL MYCELIAL FUNGI CAUSING SOFT ROT

Wood softening is the main feature of decay caused by soft rot fungi. Causative agents of soft rot are largely represented by ascomycetous and imperfect fungi (*Chaetomium globosum*, *Ch. funiculosum*, *Stachybotris atra*, *Trichoderma lignorum*, and certain species of the genera *Stysanus*, *Trichurus*, *Orbicula*, *Bispora*, *Rhizoctonia*, *Stemphylium*, and *Coniothyrium*) [20]. The absolute content of lignin in wood specimens affected by these fungi is not changed significantly. Compared to basidiomycetes, soft rot fungi are not as adapted to degrading lignin. Nevertheless, they are capable of mineralizing synthetic lignins to carbon dioxide; moreover, the transformation is not limited to methoxyls, but also includes propanoid groups and even aromatic rings [1].

Soft rot fungi release a variety of hydrolases, which cleave structural polysaccharides of cell walls, and oxidoreductases, which act both on carbohydrate components of wood (glucose oxidases, galactose oxidases, and CDHs) and phenolic compounds (peroxidases and laccases).

Ascomycetes causing wood decay and humification. Species belonging to the genera *Trichoderma* (*Hypocrea*), *Humicola* (*Scytalidium*), *Chaetomium*, *Aspergillus*, *Fusarium* (*Nectria*), and *Penicillium* are the best-known producers of cellulase–hemicellulase systems [5]. Polysaccharide hydrolase systems of ascomycetes and deuteromycetes have the same principal composition as those of the most specific xylotrophs causing white rot.

Soft rot fungi and filamentous fungi causing humification of wood debris form, in addition to glycosyl hydrolases, a series of oxidoreductases. The ability to excrete highly active sugar oxidases forming H_2O_2

(such as glucose oxidase [110] and galactose oxidase [111]) is widespread among micromycetes (representatives of the genera *Penicillium*, *Aspergillus*, and *Fusarium*). The ascomycete *Geotrichum candidum*, producing a cellulase–hemicellulase system [112], possesses an active nonspecific peroxidase affecting a broad spectrum of aromatic pigments [113, 114].

The similarity of the two types of lignocellulose degraders—specific (agaric and cap mushrooms, growing under natural conditions only on wood) and nonspecific (ascomycetous and imperfect fungi, causing natural decay of various plant substrates)—is emphasized by the observation that they share the ability to form laccases (polyphenol oxidases). It is believed that the enzymes are involved in both humus formation and ligninolysis [115–117]. Among the saprotrophic and phytoparasitic micromycetes inducing humification and forming laccases, of particular interest are *Rhizoctonia praticola* [118, 119] and the thermophilic cellulose degraders, the ascomycetes *Chaetomium thermophile* and *Myceliophthora* (*Sporotrichum*) *thermophila* [120, 121].

Unlike laccases of wood-decaying fungi, which have acidic pH optima (4.0–5.0) for oxidation of phenolic substrates, laccases of the majority of micromycetes causing humification have more neutral pH optima, e.g., 6.0 (in *M. thermophila* and *C. thermophile*) or 7.0 (in *Scytalidium thermophilum* and *Rhizoctonia solani*) [122]. In this respect, laccases of humification inducers are more similar to the enzymes of coprophilic wood-decaying species of the family Coprinaceae (*Coprinus cinereus, Panaeolus sphinctrinus, P. papilonaceus*, and *C. friesii*), which have pH optima in the range 6.5–7.5 [123], or wood enzymes involved in lignin biosynthesis, such as laccase of the lacquer tree, *Rhus vernicifera* (the pH optimum for syringaldazine oxidation is 9.0) [122], than to laccases of agaric and cap mushrooms.

The higher pH optima for polyphenol oxidation characteristic of micromycetes inducing humification (which are capable of decaying wood and other plant substrates in soil or manure) are likely related to the function of their laccases. It was shown that laccases isolated from duff (either as individual enzymes or in complex with humic substances) have pH optima in the range 5.7–7.0 [124–126].

Neutral laccases of soft rot fungi may differ from acid laccases of white rot fungi in several respects. Enzymes of the former group polymerize low-molecular-weight phenols (as do laccases of wood proper) and detoxify compounds of the natural wood defense system (lignans, catechins, tannins, and stilbenes) by their condensation (involving free carbohydrates and amino acids), resulting in humification. Enzymes of the latter group may play an important role in free-radical ligninolysis. When the process of polymerization is facilitated (e.g., at near-neutral pH values), enzymes of agaric mushrooms are less active, which proves their predisposal to degradation, rather than condensation, of substrates.

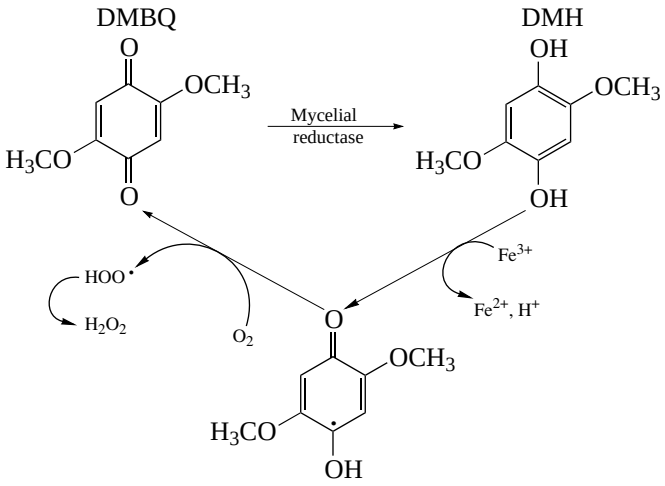

Fig. 3. Schematic representation of the putative mechanism of extracellular Fe^{3+} reduction and H_2O_2 formation (components of the Fenton reagent) in *G. trabeum* [108].

CDHs are found in *Myceliophthora thermophila* [127], *Neurospora sitophila* [128], *Chaetomium cellulolyticum* [129], and *Humicola insolens* [130]. The availability of an external CDH in soft rot fungi suggests that lignocellulose degradation in the presence of Fe^{2+} and peroxide may involve a free-radical process (in acidic medium). Of note, CDHs from soft rot fungi (e.g., *Humicola insolens*) may have neutral pH optima, which is not the case of the enzymes of basidiomycetes [13].

The formation of enzymes degrading lignocellulose in mycelial fungi may be regulated by oxidative stress in a manner similar to that described above for basidiomycetes. When grown on bagasse and sawdust under the conditions of microaerophilic or aerobic–microaerophilic fermentation, *Trichocladium canadense, Geotrichum* sp., *Fusarium* sp., and an unidentified basidiomycete formed the enzymes more readily at lower concentrations of oxygen. Of note, the soil mycelium fungus *Fusarium* sp. was comparable in activity to the basidiomycete [131].

DEGRADATION OF XENOBIOTICS

Large-scale pollution of the biosphere with condensed polycyclic hydrocarbons and aromatic compounds containing chlorine, sulfur, and nitrogen, which is attendant to diverse industrial activities of human beings (fuel utilization and energy production; paper manufacturing; production and utilization of heat carriers and dielectrics, hydraulic oils, diluents, plasticizers and additives, synthetic dyes, and plant defense agents), has resisted all countermeasures taken thus far [132].

The danger associated with the accumulation of poorly soluble and highly resistant pollutants, as well as the need for economic recovery of the polluted territories, provides a strong incentive for identifying microorganisms that would be capable of degrading the above xenobiotics; wood-decaying fungi have also been screened for such activities [133–135].

Xylotrophic basidiomycetes. White rot fungi, as well as their extracellular enzymes involved in lignin transformation (laccases, LPs, and Mn-Ps), are capable of oxidizing and degrading in vitro a broad range of xenobiotics: polycyclic aromatic hydrocarbons (anthracene, benz[a]pyrene, naphthalene, and phenanthrene) [136–141]; polychlorinated phenols (2,4-di-, 2,4,5- and 2,4,6-tri-, and pentachlorophenols), chlorinated guaiacol and benzoate derivatives, 2,4,6-trichlorophenoxyacetate, and chlorinated biphenyls [142–146]; lignosulfonates and chlorolignins, stable polymers (polyacrylate, polyacrylamide, polycaprolactam, and polyethylene), 2,4-dichloroaniline, dioxins, explosives nitrates, dyes, and pesticides (atrazine, DDT, chlordane, Lindane, Toxaphene, and endosulfan) [147–149].

When grown in liquid media, white rot fungi *Phanerochaete chrysosporium* and *Trametes versicolor* convert diketonitrile (a soil transformation product of the herbicide isoxaflutol) to benzoate with yields of 24.6 and 15.1%, respectively, over a period of 12–15 days. The synthesis of the oxidative enzymes of the fungi is synchronous with the diketonitrile degradation. The xenobiotic is also transformed by the purified laccase (in the presence of ABTS at pH 3.0) [150].

The multiple-step process of pentachlorophenol degradation by the fungus *P. chrysosporium* occurring under the conditions of secondary metabolism caused by nitrogen deficiency includes LP- or Mn-P-catalyzed oxidative dehalogenation (with the formation of tetrachloro-1,4-benzoquinone) and several stages of reducing dehalogenation and hydroxylation. Unlike oxidative dehalogenation, which occurs only during secondary metabolism, subsequent processes are characteristic of both secondary and primary fungal metabolism. As a result, tetrachloro-1,4-benzoquinone is degraded via two parallel pathways, sharing an intermediate. In the first pathway, it is reduced to tetrachlorohydroquinone, which loses all four chlorine atoms in the course of subsequent reduction, forming 1,4-hydroquinone. The latter is then hydroxylated, with the formation of 1,2,4-trihydroxybenzene. The alternative pathway includes the conversion of tetrachloro-1,4-benzoquinone (enzymatic or nonenzymatic) to 2,3,5-trichlorotrihydroxybenzene, which also loses chlorine atoms in the course of subsequent reduction until 1,2,4-trihydroxybenzene, the common aromatic end product of both pathways, is formed. It is further metabolized to carbon dioxide via cleavage of the aromatic cycle, which requires elimination of all five chlorine atoms [151].

Reductive dehalogenation of tetrachloro-1,4-hydroquinone (TCHQ), resulting in the formation of trichlorohydroquinone, involves a membrane glutathione transferase, which is highly specific for glutathione. This enzyme forms a glutathione conjugate of TCHQ (GS–TCHQ). At the second stage of the process, a cytosolic reductase converts GS–TCHQ to TCHQ in the presence of reduced glutathione, cysteine, or dithiothreitol. This pair of enzymes effects reductive dehalogenation of TCHQ and 2,6-dichlorohydroquinone, intermediates of chlorophenol transformation by ligninolytic fungi [152].

Quinone reduction is effected by 1,4-benzoquinone reductase; in *P. chrysosporium*, this flavine enzyme is either intracellular or membrane-associated. It catalyzes NADH oxidation by 2,6-dimethoxy-1,4-benzoquinone with the formation of hydroquinones (stoichiometry, 1 : 1). Quinones formed by autoxidation of the unstable 1,2,4-trihydroxybenzene and 5-chloro-2,3,4-trihydroxybenzene are also reduced by the enzyme [153]. The enzyme reduces cation radicals formed in the course of catalysis by LP or Mn-P, thereby protecting the fungal cells from oxidative damage [154].

Similar to the process of ligninolysis, certain enzymes synthesized under conditions of oxidative stress are involved in degradation of organic xenobiotics. The white rot fungus *Funalia trogi* and the yeast *Kluyveromyces marxianus* respond to paraquat by an increase in the activities of glutathione reductase and SOD; in contrast, the formation of catalase is suppressed [155, 156].

The capacity of white rot fungi for degrading aromatic pesticides—diuron, methalaxyl, atrazine, and terbutylazine (Fig. 4)—is not correlated with their activity in the Poly R-478 decoloration test (which is used as an indicator of ligninolytic activity). On the other hand, degradation rates of various herbicides are in agreement with each other. *Coriolus versicolor, Hypholoma fasciculare*, and *Stereum hirsutum*, which degrade more than 86% of diuron, atrazine, and terbutylazine in 6 weeks, are the most active in this respect (of note, less than 44% of methalaxyl is degraded under the same conditions). When tested in the field (growth on an organic substrate), *C. versicolor* and *H. fasciculare* degraded 30% of chloropyriphos in 6 weeks; unexpectedly, *S. hirsutum*, which showed maximum activity under laboratory conditions, was virtually inactive [157].

Extracellular CDH, membrane methyl transferases, and cytochrome P-450 monooxygenase are also involved in degradation of the most resistant xenobiotics by white rot fungi. Degradation of atrazine by the basidiomycete *Pleurotus pulmonarius* involves lipoxygenase, peroxidase, and cytochrome P-450 [158]. Mn^{2+}, which activates these enzymes, stimulates atrazine transformation to *N*-dealkylated and propylhydroxylated metabolites. Conversely, antioxidants and inhibitors of lipoxygenase and peroxidase (nordihydroguaiaretic acid) or cytochrome P-450 (piperonyl butoxide) suppress atrazine transformation by the fungus.

Ligninolytic fungi degrade a variety of polyaromatic xenobiotics through the involvement of lipoper-

oxide free radicals (generated by autoxidation of polyunsaturated fatty acids) [85, 86]. Reactions of degradation of polycyclic aromatic structures and other highly resistant aromatic xenobiotics initiated by Mn-P producer strains in the presence of Mn^{2+} and polyunsaturated fatty acids (or derivatives thereof) appear to follow the same mechanism [136, 159]. The culture of *P. chrysosporium* and its Mn-P catalyze lipoperoxide degradation of condensed aromatic compounds containing three to six cycles. Polycyclic aromatic hydrocarbons interacting with free radicals are the most susceptible to degradation [160]. Extracellular LPs of ligninolytic basidiomycetes directly oxidize condensed polycyclic aromatic hydrocarbons, whereas Mn-P achieves this effect by inducing lipid peroxidation [161].

The ability of Mn-P to degrade xenobiotics via free-radical intermediates is not limited to oxidation of polyunsaturated fatty acids. Other examples of mineralization mediated by this enzyme include such stable xenobiotics as pyrene, anthracene, benz[a]pyrene, benz[a]anthracene, and phenanthrene [162]. In all these cases, reduced glutathione, which forms thiyl radicals under the effect of Mn-P, acts a mediator.

In the presence of an artificial mediator, 1-hydroxybenzotriazole, laccase induces the formation of linoleic acid hydroperoxide and unsaturated lipids (Mn^{2+} and H_2O_2 are not involved in this process). The system degrades condensed aromatic xenobiotics, such as phenanthrene; phananthrene-9,10-quinone and 2,2'-diphenic acid are the major transformation products [138]. Laccase of *Coriolus versicolor* dechlorinates chlorophenols [6].

A highly efficient chlorophenol degrader is the basidiomycete *Panus tigrinus*. This fungus, which forms endogenous laccase mediators, and its extracellular laccases eliminate up to 0.4 g/l di- and trichlorophenols from culture medium or wastewater. Trichlorophenol degradation by laccases involves the formation of dichlorocatechol (by *ortho*-dehalogenation) [6].

On the contrary, brown rot fungi may degrade chlorophenols using nonenzymatic mechanisms of hydroxyl radical formation, which involve quinone intermediates [107]. Brown rot fungi *Gloeophyllum trabeum* and *G. striatum*, which form neither laccases, LPs, nor Mn-Ps, also degrade quinone-forming xenobiotics. Thus, degradation of pentachlorophenol and 2,4-dichlorophenol by these fungi follows the Fenton mechanism, regardless of whether they are grown on wheat straw or under conditions ruling out the involvement of CDH, glucose oxidase, or like enzymes. *G. striatum* causes oxidative decarboxylation and hydroxylation of aromatic compounds [163]. Degradation of chlorophenols by *G. striatum* is most efficient in mineral medium in the absence of other sources of carbon, nitrogen, and phosphate [143]. Degradation of 2,4-dichlorophenol by *G. striatum* involves the formation of 4-catechol and 3,5-dichlorocatechol intermediates, whereas carbon dioxide is the end product; the same

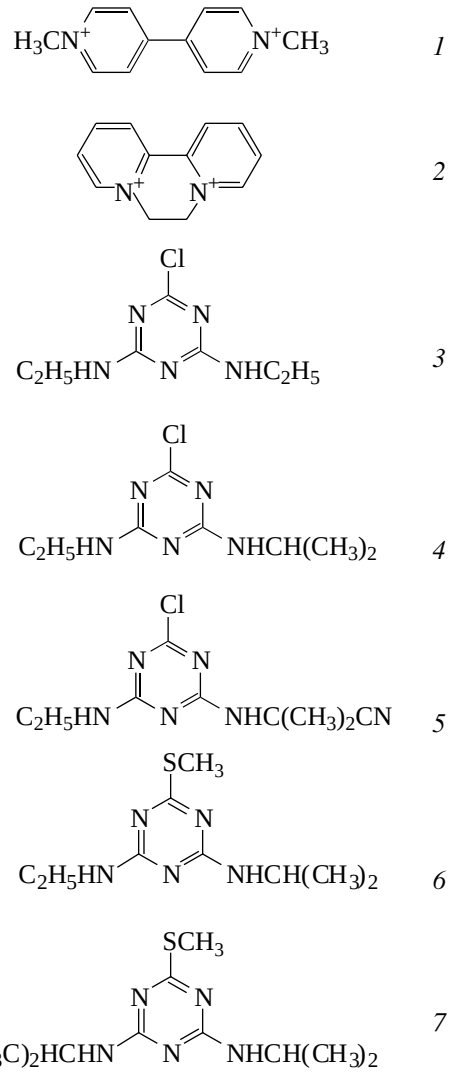

Fig. 4. Structural formulae of degradation-resistant pesticides transformed by fungi: (*1*) paraquat; (*2*) diquat; (*3*) simazine; (*4*) atrazine; (*5*) cyanazine; (*6*) ametrine; (*7*) prometrine.

results were obtained under the effect of the system $Fe^{2+} + H_2O_2$ [164].

Soil fungi (including thermophiles of compost) that cause humification of organic residues seem to be involved in detoxification of xenobiotics in lands of industrial and agricultural utilization [115, 165].

Soil yeast *Lipomyces starkeyi* grow well in media containing 1,3,5-triazine or cyanuric acid as the source of nitrogen. Simazine, atrazine, cyanazine, ametrine, and prometrine are also assimilated by *L. starkeyi*, whereas melamine and thiocyanuric acid inhibit the growth of the yeast and decrease the size of its colonies [166].

Certain imperfect fungi are capable of deactivating xenobiotics (via effects of intracellular hydroxylases) [167, 168].

A mixed culture of fungi isolated from the soil of a metholachlor-polluted field degrades 50% of this xenobiotic in 3.5 days. The main active species in the culture are *Aspergillus flavus* and *A. terricola*. The composition of degradation products indicates that the xenobiotic is subjected to hydrolytic dechlorination, *N*-dealkylation, and amide bond cleavage. Among the new products identified are 6-methyl-2-ethylacetanilide and 6-methyl-2-ethylanilide. The mixed culture degrades 99% of metholachlor at initial concentrations of up to 100 mg/l [169].

Species introduced into soil for the purpose of its remediation include pesticide degrader fungi known to be predominant in the rhizosphere, which are isolated under laboratory conditions. The fungus *Trichoderma harzianum* 2023, used as a biocontrol agent, is resistant to many pesticides; when grown in liquid culture, this species actively transforms pentachlorophenol into pentachloroanisole [170].

Fungi isolated from pesticide-polluted soils (263 cultures) or soils (in a suburb of Annaba, Algeria) not subjected to pollution (288 cultures) were compared for the ability to degrade metamitron, metribuzin, linuron, and metobromuron. The species encountered most frequently—*Aspergillus fumigatus, A. niger, A. terreus, Absidia corymbifera,* and *Rhizopus microsporus* var. *microsporus*—were insensitive to the pesticides. Herbicides inhibited the fungi of the genus *Trichoderma,* but stimulated those of the genera *Absidia* and *Fusarium*. Out of the 53 species tested in liquid media, only the well-known laccase producer *Botrytis cinerea* isolated from pollutant-free soils, as well as *Sordaria superba* and *Absidia fusca* isolated from polluted soils, eliminated more than 50% of metribuzin in 5 days. Metamitron was the most degradation-resistant compound. Analysis of 21 fungal species demonstrated that only *Alternaria solani* (pollutant-free soils), *Drechsleria australiensis* (both types of soils), and *Absidia fusca* (polluted soils) eliminated 10–16% of this compound. Out of the 12 species grown on linuron, 7 were incapable of degrading this pollutant. Two strains of *Sordaria macrospora* eliminated 22–25% of the compound, whereas *Botrytis cinerea* exhibited the capacity for near-complete assimilation. Out of the 31 species tested for the ability to eliminate metobromuron, only one fungus (the phytopathogen *Botrytis cinerea* from either type of soils) was capable of complete assimilation; *Rhizopus oryzae* and *Absidia fusca* isolated from polluted soils eliminated 40 and 47% of the compound, respectively. No clear-cut correlation could be established between the degree of soil pollution and the ability of the fungi to degrade the pesticides. *Absidia fusca* and *Botrytis cinerea*, capable of degrading three out of the four compounds tested, showed significant promise as agents for soil remediation [171].

The number and composition of microorganisms in soils subjected to large-scale treatment with dioxin-containing defoliants during the Vietnam War were analyzed in detail in [172]. At present, the main components of Agent Orange—2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid—are no longer found in the soils; nevertheless, technical admixtures, such as the highly resistant hepta- and octachlorobenzodioxins, are still orders of magnitude in excess of the maximum permissible concentrations. Changes in the composition of populations of soil microorganisms were studied by analyzing specimens of soil differing in the levels of dioxin pollution. Soils containing 5–7 ng/kg dioxins were characterized by reduced numbers of fungi (actinomycetes were less sensitive in this respect). At average pollution levels of 10–250 ng/kg, the diversity of fungal species was strongly affected, although the number of fungi in the population did not change; this observation suggests that only the resistant species were selected. In heavily polluted specimens (1500–100000 ng/kg), all groups of microorganisms were suppressed, to the point of complete elimination of the fungal component of the microbiota.

Humic and fulvic acids play the greatest role in immobilization of phenolic xenobiotics in soil [173]. Binding of these agents to a xenobiotic or its transformation product has the same nature as the process of humification. This detoxification mechanism was first demonstrated in the phytopathogenic fungus *Rhizoctonia praticola,* a representative of fungi with sterile mycelia and an active laccase producer. The ability of oxidoreductases oxidizing phenolic substrates to detoxify aromatic compounds (through their condensation with humic substances, fulvic acids, or low-molecular-weight precursors thereof) has been demonstrated for 2,4-dichlorophenol, pentachlorophenol [174, 175], and chloroanilines [176]. Even those xenobiotics that are not the substrates of these enzymes undergo transformation in the presence of certain cosubstrates. Thus, cyprodinil, a broad-spectrum fungicide resistant to transformation by laccase, was efficiently bound to natural phenolic mediators (particularly, phenols carrying one or two methoxy substituents). Labeled cyprodinil was preferentially detected in dark brown polymerization products precipitated from the reaction medium, which appeared as cross-linked oligomers of cyprodinil and the mediator. Humic acid salts either inhibited the elimination of the fungicide (in the presence of the most active mediators) or stimulated its binding and elimination (in the presence of less active mediators or in mediator-free media) [177]. Peroxidase exerts similar effects: on addition of H_2O_2, the enzyme polymerizes phenol, *ortho*-cresol, and 2,4-dichlorophenol, incorporating these xenobiotics into the fraction of humic and fulvic acids, as well as into the insoluble soil humus [178].

Our data [179, 180] demonstrate that atrazine consumption by soil fungi is not correlated with their laccase activity. The laccase-free nonsporulating mycelial fungus INBI 2-26(–), isolated from dioxin-containing soils of South Vietnam and being a CDH producer, exhibits the same capacity for atrazine consumption as

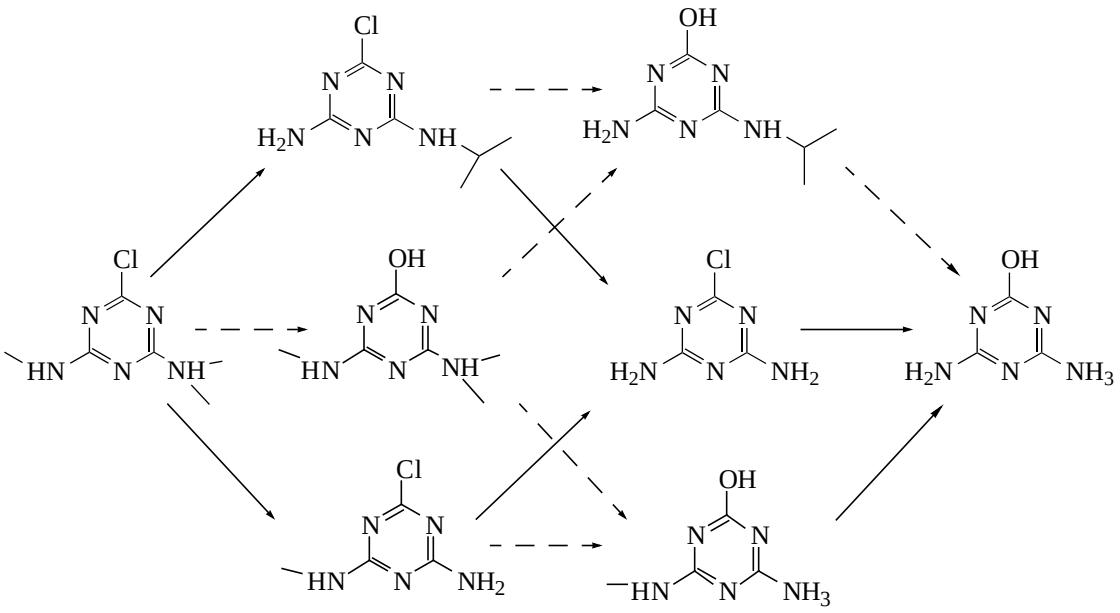

Fig. 5. Atrazine degradation pathway.

the laccase producer INBI 2-26(+), isolated from the same soils.

Similar to dioxins, the ability of atrazine to decrease the number of soil microorganisms is more pronounced in the case of fungi as compared to bacteria (PCR data on the ratio of 18- and 16S rDNA in soil specimens) [182]. Thus, only the most resistant forms of fungi survive in the presence of the herbicide. Of note, the relative contributions of bacteria and fungi to atrazine degradation are approximately equal to each other (results of experiments in which each population was selectively suppressed by specific inhibitors) [183].

The mechanisms of atrazine and dioxin degradation by fungi were largely studied in wood-decaying basidiomycetes of the genera *Phanerochaete*, *Pleurotus*, *Coriolus*, *Cerrena*, *Trappea*, and *Phlebia*, each of which produces a set of oxidases adapted to degradation of phenylpropanoid structures of lignin in an acidic medium [180, 184–188]. Data on the degradation of atrazine and related triazine herbicides by soil mycelial fungi are scarce. An unidentified ligninolytic fungus has been isolated from dioxin-containing soils that is capable of degrading dioxin and its analogues, dibenzofurans, and is at least as efficient in this respect as the white rot fungi *P. chrysosporium, Pleurotus florida*, and *Dichomitus squalens* [189]. The list of atrazine degrader cultures described by us [181] can be supplemented by a single case reported in the literature. The soil fungus *Penicillium steckii* assimilates simazine, an atrazine-related compound, although the underlying mechanism remains to be elucidated [190]. Atrazine consumption by the fungi *Emericella nidulans* and *Penicillium miczynskii* is believed to involve irreversible adsorption of the herbicide by mycelia [191]. In our experiments, however, no appreciable mycelial adsorption of atrazine by the cultures studied was observed. It was noted that atrazine detoxification by a consortium of soil fungi is achieved via the formation of its dealkylated derivatives, without cleavage of the triazine ring [192]. It is worth mentioning in this connection that atrazine degradation by the system Fe^{2+} + H_2O_2 also involves dealkylation [193, 194] (Fig. 5). A mechanism of biodegradation described in the literature involves generation of hydroxyl radicals in the Fenton reaction. This mechanism is believed to operate in basidiomycetes, such as the brown rot fungus *Coniophora puteana* [99]. Similar to the strain 2-26(–), this fungus is a CDH producer. It remains for further research, however, to explore the possibility of CDH involvement in atrazine degradation.

In conclusion, we have analyzed recent reports on the transformation of natural and synthetic aromatic compounds by basidiomycetes and soil mycelial fungi.

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REFERENCES

1. Rabinovich, M.L., Bolobova, A.V., and Kondrashchenko, V.I., *Teoreticheskie osnovy biotekhnologii drevesnykh kompozitov. Drevesina i razrushayushchie ee griby* (Theoretical Basis of the Biotechnology of Wood Composites. Wood and Wood-decaying Fungi), Moscow: Nauka, 2001, vol. 1.
2. Rodriguez, J., Ferraz, A., and Nogueira, R.F., *Appl. Biochem. Biotechnol.*, 1997, vol. 62, no. 2-3, pp. 233–234.
3. Bergbauer, M.S. and Newell, Y., *FEMS Microbiol.*, 1992, vol. 86, pp. 341–348.
4. Bolobova, A.V., Askadskii, A.A., Kondrashchenko, V.I., and Rabinovich, M.L., *Teoreticheskie osnovy biotekhnologii drevesnykh kompozitov. Kn.II. Fermenty, modeli, protsessy* (Theoretical Basis of the Biotechnology of Wood Composites: II. Enzymes, Models, and Processes), Moscow: Nauka, 2002, vol. 2.
5. Rabinovich, M.L., Mel'nik, M.S., and Bolobova, A.V., *Prikl. Biokhim. Mikrobiol.*, 2002, vol. 38, no. 4, pp. 355–373.
6. Leontievsky, A.A., Ligninases of Basidiomycetes, *Doctoral (Biol.) Dissertation*, Pushchino: Institut mikrobiologii i fiziologii mikroorganizmov RAN, 2002.
7. Berger, R.G., Neuhauser, K., and Drawert, F., *Flavour Fragrance J.*, 1986, vol. 1, pp. 181–185.
8. Gallois, A., Gross, B., Langlois, D., *et al.*, *Mycol. Res.*, 1990, vol. 4, pp. 494–504.
9. Lapadatescu, C., Feron, G., Vergoignan, C., *et al.*, *Appl. Microbiol. Biotechnol.*, 1997, vol. 47, pp. 708–714.
10. Lomascolo, A., Lesage-Meessen, L., and Labat, M., *Can. J. Microbiol.*, 1999, vol. 45, no. 8, pp. 653–657.
11. Bonnina, E., Brunel, M., and Gouy, Y., *Enzyme Microb. Technol.*, 2001, vol. 28, no. 1, pp. 70–80.
12. Reddy, C.A., *J. Biotechnol.*, 1993, vol. 30, no. 1, pp. 91–107.
13. Staszczak, M., Zdunek, E., and Leonowicz, A., *J. Basic Microbiol.*, 2000, vol. 40, no. 1, pp. 51–63.
14. Muheim, A., Waldner, R., Leisola, M.S.A., and Fiechter, A., *Enzyme Microb. Technol.*, 1990, vol. 12, pp. 204–209.
15. Guillén, F., Martinez, A.T., Martinez, M.J., and Evans, C.S., *Appl. Microbiol. Biotechnol.*, 1994, vol. 41, pp. 465–470.
16. Gutierrez, A., Caramelo, L., Prieto, A., *et al.*, *Appl. Environ. Microbiol.*, 1994, vol. 60, pp. 1783–1788.
17. Muheim, A., Waldner, R., Sanglard, D., *et al.*, *Eur. J. Biochem.*, 1991, vol. 195, pp. 369–375.
18. Sethuraman, A., Akin, D.E., Eisele, J.G., and Eriksson, K.-E.L., *Can. J. Microbiol.*, 2000, vol. 44, pp. 872–885.
19. Mester, T., Swarts, H.J., and Romero Sole, S., *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 1987–1994.
20. Ripachek, V., *Biologiya derevorazrushayushchikh gribov* (Biology of Wood-Degrading Fungi), Moscow: Lesnaya promyshlennost', 1967.
21. De Jong, E., Field, J.A., and Dings, J.A., *FEBS Lett.*, 1992, vol. 305, no. 3, pp. 220–224.
22. Field, J.A., Verhagen, F.J., and De Jong, E., *Trends Biotechnol.*, 1995, vol. 13, pp. 451–456.
23. Spinnler, H.-E., De Jong, E., and Mauvais, G., *Appl. Microbiol. Biotechnol.*, 1994, vol. 42, pp. 212–221.
24. Swarts, H.J., Verhagen, F.J.M., Field, J.A., and Wijnberg, J.B., *Phytochemistry*, 1996, vol. 42, pp. 1699–1701.
25. Teunissen, P.J.M., Swarts, H.J., and Field, J.A., *Appl. Microbiol. Biotechnol.*, 1997, vol. 47, pp. 695–700.
26. Hjelm, O., Boren, H., and Asplund, G., *Chemosphere*, 1996, vol. 32, pp. 1719–1728.
27. Teunissen, P.J. and Field, J.A., *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 3, pp. 830–835.
28. Valli, K. and Gold, M.H., *J. Bacteriol.*, 1991, vol. 173, pp. 345–352.
29. Pickard, M.A., Vandertol, H., Roman, R., and Vǎzquez-Duhalt, R., *Can. J. Microbiol.*, 2000, vol. 45, pp. 627–631.
30. Bonnarme, P. and Jefris, T.W., *Appl. Environ. Microbiol.*, 1990, vol. 56, no. 1, pp. 210–217.
31. Bogan, B.W. and Lamar, R.T., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 5, pp. 1597–1603.
32. Camarero, S., Ruiz-Duenas, F.J., Sarkar, S., *et al.*, *FEMS Microbiol. Lett.*, 2000, vol. 191, no. 1, pp. 37–43.
33. Mester, T. and Field, J.A., *J. Biol. Chem.*, 1988, vol. 273, no. 25, pp. 15412–15417.
34. Leisola, M., Ulmer, D., and Fiechter, A., *Eur. J. Appl. Microbiol. Biotechnol.*, 1983, vol. 17, pp. 113–116.
35. Michel, F.C., Grulcke, E.A., and Reddy, C.A., *Appl. Environ. Microbiol.*, 1992, vol. 58, pp. 1740–1745.
36. Zacchi, L., Burla, G., Zuolong, D., and Harvey, P.J., *J. Biotechnol.*, 2000, vol. 78, no. 2, pp. 185–192.
37. Zacchi, L., Morris, I., and Harvey, P.J., *Microbiology*, 2000, vol. 146, Pt. 3, pp. 759–765.
38. Zacchi, L., Palmer, J.M., and Harvey, P.J., *FEMS Microbiol. Lett.*, 2000, vol. 183, no. 1, pp. 153–157.
39. Perez, J. and Jeffries, T.V., *Appl. Environ. Microbiol.*, 1990, vol. 56, pp. 1806–1812.
40. Cheton, P.L.-B. and Archibald, F.S., *Free Radical Biol. Med.*, 1988, vol. 5, pp. 325–333.
41. Coassin, M., Ursini, F., and Bindoli, A., *Arch. Biochem. Biophys.*, 1992, vol. 299, pp. 330–333.
42. Tampo, Y. and Yonaha, M., *Free Radical Biol. Med.*, 1992, vol. 13, pp. 115–120.
43. Dosoretz, C.G., Rothschild, N., and Hadar, Y., *Appl. Environ. Microbiol.*, 1993, vol. 59, pp. 1919–1926.
44. Mester, T., Jong, E., and Field, J.A., *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 1881–1887.
45. Morpeth, F.F., *J. Gen. Microbiol.*, 1987, vol. 133, pp. 3521–3525.
46. Greene, R.V. and Gould, J.M., *Biochem. Biophys. Res. Commun.*, 1983, vol. 117, p. 1, pp. 275–281.
47. Li, D., Alic, M., Brown, J.A., and Gold, M.H., *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 341–345.
48. Brown, J.A., Alic, M., and Gold, M.H., *J. Bacteriol.*, 1991, vol. 173, pp. 4101–4106.
49. Périé, F.H. and Gold, M.H., *Appl. Environ. Microbiol.*, 1991, vol. 57, pp. 2240–2245.
50. Srinivasan, C., D'Souza, T.M., Boominathan, K., and Reddy, C.A., *Appl. Environ. Microbiol.*, 1995, vol. 61, pp. 4274–4277.
51. D'Souza, T.M., Merritt, C.S., and Reddy, C.A., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 12, pp. 5307–5313.

52. D'Souza, T.M., Boominathan, K., and Reddy, C.A., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 10, pp. 3739–3744.
53. Buswell, J.A., Cai, Y.J., and Chang, S.T., *FEMS Microbiol.*, 1995, vol. 128, pp. 81–88.
54. Sethuraman, A., Akin, D.E., and Eriksson, K.E., *Appl. Microbiol. Biotechnol.*, 1999, vol. 52, no. 5, pp. 689–697.
55. Reshetnikova, I.A., *Destruktsiya lignina ksilotrofnymi makromitsetami* (Destruction of Lignin by Xylotrophic Macromycetes), Moscow: Moscow State Univ., 1997.
56. Boominathan, K. and Reddy, C.A., *Proc. Natl. Acad. Sci. USA*, 1992, vol. 89, pp. 5586–5590.
57. Rabinovich, M.L., Mel'nik, M.S., and Bolobova, A.V., *Biokhimiya* (Moscow), 2002, vol. 67, no. 8, pp. 407–413.
58. Ander, P. and Marzullo, L., *J. Biotechnol.*, 1997, vol. 53, no. 2-3, pp. 115–131.
59. Henriksson, G., Johansson, G., and Pettersson, G., *J. Biotechnol.*, 2000, vol. 78, pp. 93–113.
60. Cameron, M.D. and Aust, S.D., *Enzyme Microb. Technol.*, 2001, vol. 28, no. 2-3, pp. 129–138.
61. Roy, B.P., Paice, M.G., and Archibald, F.S., *J. Biol. Chem.*, 1994, vol. 269, pp. 19745–19750.
62. Hüttermann, A. and Noelle, A., *Holzforschung*, 1982, vol. 36, pp. 283–286.
63. Fang, J., Liu, W., and Gao, P.J., *Arch. Biochem. Biophys.*, 1998, vol. 353, pp. 37–46.
64. Roy, B.P., Dumonceaux, T., Koukoulas, A.A., and Archibald, F.S., *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 4417–4427.
65. Temp, U. and Eggert, S., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 2, pp. 389–395.
66. Eggert, C., Temp, U., Dean, J.F.D., and Eriksson, K.-E.L., *FEBS Lett.*, vol. 391, pp. 144–148.
67. Christen, S., Southwell-Keely, P., and Stocker, R., *Biochemistry*, 1992, vol. 31, pp. 8090–8097.
68. Toussaint, O. and Lerch, K., *Biochemistry*, 1987, vol. 26, pp. 8568–8571.
69. Leonowicz, A., Matuszewska, A., Luterek, J., *et al.*, *Fungal Genet. Biol.*, 1999, vol. 27, no. 2-3, pp. 175–185.
70. Tanaka, H., Itakura, S., and Enoki, A., *J. Biotechnol.*, 1999, vol. 75, no. 1, pp. 57–70.
71. Hatakka, A., *FEMS Microbiol. Rev.*, 1994, vol. 13, pp. 125–135.
72. Boyle, C.D., Kropp, B.R., and Reid, I.D., *Appl. Environ. Microbiol.*, 1992, vol. 58, pp. 3217–3224.
73. Wariishi, H., Valli, K., and Gold, M.H., *Biochem. Biophys. Res. Commun.*, 1991, vol. 176, pp. 269–275.
74. Wariishi, H., Valli, K., and Gold, M.H., *J. Biol. Chem.*, 1992, vol. 267, pp. 23688–23695.
75. Perez, J. and Jeffries, T.V., *Appl. Environ. Microbiol.*, 1992, vol. 58, pp. 2402–2409.
76. Shimada, M., Akamatsu, Y., and Tokimatsu, T., *J. Biotechnol.*, 1997, vol. 53, pp. 103–113.
77. Khindaria, A., Grover, T.A., and Aust, S.D., *Arch. Biochem. Biophys.*, 1994, vol. 314, no. 2, pp. 301–306.
78. Hofrichter, M., Vares, T., and Kalsi, M., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 5, pp. 1864–1870.
79. Vyas, B.R.M., Volc, J., and Sasek, V., *Folia Microbiol.*, 1994, vol. 39, pp. 235–240.
80. Hofrichter, M., Scheibner, K., Schneegaβ, I., and Fritsche, W., *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 399–404.
81. Moen, M.A. and Hammel, K.E., *Appl. Environ. Microbiol.*, 1994, vol. 60, pp. 1956–1961.
82. Kapich, A., Hofrichter, M., Vares, T., and Hatakka, A., *Biochem. Biophys. Res. Commun.*, 1999, vol. 259, no. 1, pp. 212–219.
83. Watanabe, T., Katayama, S., Enoki, M., *et al.*, *Eur. J. Biochem.*, 2000, vol. 267, no. 13, pp. 4222–4231.
84. Bao, W., Fukushima, Y., Jensen, K.A., *et al.*, *FEBS Lett.*, 1994, vol. 354, no. 3, pp. 297–300.
85. Kapich, A.N., Jensen, K.A., and Hammel, K.E., *FEBS Lett.*, 1999, vol. 461, no. 1-2, pp. 115–119.
86. Kapich, A.N. and Shishkina, L.N., *Mikol. fitopatol*, 1992, vol. 26, no. 6, pp. 486–492.
87. Denisova, N.P., Proteolytic Enzymes of Basidiomycetes, Taxonomic and Ecological Aspects of Their Study, *Doctoral (Biol.) Dissertation*, Leningrad: Botanicheskii Institut RAN, 1991.
88. Bourbonnais, R. and Paice, M.J., *FEBS Lett.*, 1990, vol. 267, pp. 99–102.
89. Guillen, F., Gomez-Toribio, V., Martinez, M.J., and Martinez, A.T., *Arch. Biochem. Biophys.*, 2000, vol. 383, no. 1, pp. 142–147.
90. Leontievsky, A., Myasoedova, N., Pozdnyakova, N., and Golovleva, L., *FEBS Lett.*, 1997, vol. 413, no. 3, pp. 446–448.
91. Leontievsky, A.A., Vares, T., Lankinen, P., *et al.*, *FEMS Microbiol. Lett.*, 1997, vol. 156, no. 1, pp. 9–14.
92. Call, H.P. and Mücke, I., *J. Biotechnol.*, 1997, vol. 53, pp. 163–202.
93. McFee, W.W. and Stone, E.L., *Soil Sci. Soc. Am. Proc.*, 1966, vol. 30, pp. 513–516.
94. Wetzstein, H.-G., Schmeer, N., and Karl, W., *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 4272–4281.
95. Thorn, R.G., Malloch, D.W., and Ginns, J., *Can. J. Bot.*, 2000, vol. 76, pp. 686–693.
96. Bhattacharjee, B., Roy, A., and Majumder, A.L., *Biochem. Mol. Biol. Int.*, 1993, vol. 6, pp. 1143–1152.
97. Chrapkowska, K.J., *Acta Microbiol. Pol.*, 1984, vol. 33, no. 2, pp. 137–145.
98. Schmidhalter, D.R. and Canevascini, G., *Arch. Biochem. Biophys.*, 1993, vol. 300, no. 2, pp. 559–563.
99. Hyde, S.M. and Wood, P.M., *Microbiology*, 1997, vol. 143, p. 259.
100. Eriksson, K.-E., Blanchette, L., and Ander, P., *Microbial and Enzymatic Degradation of Wood and Wood Components*, New York: Springer, 1990.
101. Bringer, S., Sprey, B., and Sahm, H., *Eur. J. Biochem.*, 1979, vol. 101, no. 2, pp. 563–570.
102. Koenigs, J.W., *Wood Fiber*, 1974, vol. 6, pp. 66–80.
103. Goodell, B., Jellison, J., Liu, J., *et al.*, *J. Biotechnol.*, 1997, vol. 53, pp. 133–162.
104. Hirano, T., Tanaka, H., and Enoki, A., *Holzforschung*, 1997, vol. 51, pp. 389–395.
105. Enoki, A., Itakura, S., and Tanaka, H., *J. Biotechnol.*, 1997, vol. 53, pp. 265–272.
106. Qian, S.Y. and Buettner, G.R., *Free Radic. Biol. Med.*, 1999, vol. 26, no. 11, pp. 1447–1456.

107. Zhu, B.Z., Kitrossky, N., and Chevion, M., *Biochem. Biophys. Res. Commun.*, 2000, vol. 270, no. 3, pp. 942–946.
108. Kerem, Z., Jensen, K.A., and Hammel, K.E., *FEBS Lett.*, 1999, vol. 446, no. 1, pp. 49–54.
109. Paszczynski, A., Crawford, R., Funk, D., and Goodell, B., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 2, pp. 674–679.
110. Bilai, V.I., Nikol'skaya, O.O., and Pidoplichko, M.M., *Mikrobiol. zhurn.*, 1968, vol. 30, no. 5, pp. 418–424.
111. Koroleva, O.V., Rabinovich, M.L., Buglova, T.T., and Yaropolov, A.I., *Prikl. Biokhim. Mikrobiol.*, 1983, vol. 19, no. 5, pp. 632–637.
112. Rodionova, N.A., Tiunova, N.A., Feniksova, R.V., *et al.*, *Dokl. Akad. Nauk SSSR, ser. Biokhimiya*, 1974, vol. 214, no. 5, pp. 1206–1209.
113. Kim, S.J. and Shoda, M., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 3, pp. 1029–1035.
114. Sugano, Y., Nakano, R., Sasaki, K., and Shoda, M., *Appl. Environ. Microbiol.*, 2000, vol. 66, no. 4, pp. 1754–1758.
115. Chefetz, B., Chen, Y., and Hadar, Y., *Appl. Environ. Microbiol.*, 1998, vol. 64, no. 9, pp. 3175–3179.
116. Stevenson, F.J., *Humus Chemistry*, New York: Wiley, 1994.
117. Varadashari, C. and Ghosh, K., *Plant Soil*, 1984, vol. 77, pp. 305–313.
118. Bollag, J.M., Sjoblad, R.D., and Liu, S.Y., *Can. J. Microbiol.*, 1979, vol. 25, no. 2, pp. 229–233.
119. Bollag, J.M., Shuttleworth, K.L., and Anderson, D.H., *Appl. Environ. Microbiol.*, 1988, vol. 54, no. 12, pp. 3086–3091.
120. Chefetz, B., Kerem, Y., Chen, Y., and Hadar, Y., *Soil Biol. Biochem.*, 1998, vol. 30, pp. 1091–1098.
121. Berka, R.M., Schneider, P., and Golightly, F., *Appl. Environ. Microbiol.*, 1997, vol. 63, no. 8, pp. 3151–3157.
122. Xu, F., Shin, W., and Brown, S.H., *Biochim Biophys. Acta*, 1996, vol. 1292, pp. 303–311.
123. Heinzkill, A., Schneider, P., Caspersen, M., *et al.*, *Enzyme Microb. Technol.*, 1999, vol. 25, pp. 502–508.
124. Ruggiero, P. and Radogna, V.M., *Soil Sci.*, 1984, vol. 138, pp. 74–87.
125. Suflita, J.M. and Bollag, J.M., *Soil Biol. Biochem.*, 1980, vol. 24, pp. 743–749.
126. Criquet, S., Tagger, S., Vogt, G., *et al.*, *Soil Biol. Biochem.*, 1999, vol. 31, pp. 1239–1244.
127. Canevascini, G., *Methods Enzymol.*, 1988, vol. 160, pp. 443–448.
128. Dekker, R.F.H., *J. Gen. Microbiol.*, 1980, vol. 120, pp. 309–316.
129. Fähnrich, P. and Irrgang, K., *Biotechnol. Lett.*, 1982, vol. 12, pp. 775–780.
130. Schou, C., Christensen, M.H., and Schulein, M., *Biochem. J.*, 1998, vol. 330, pp. 565–571.
131. Pavarina, E.C. and Durrant, L.R., *Appl. Biochem. Biotechnol.*, 2002, vol. 98–100, pp. 663–677.
132. Abramowicz, D.A., *Crit. Rev. Biotechnol.*, 1990, vol. 10, pp. 241–251.
133. Bumpus, J.A., Tien, M., Wright, D., and Aust, S.D., *Science* (Washington, D.C.), 1985, vol. 228, pp. 1434–1436.
134. Aust, S.D., *Microb. Ecol.*, 1990, vol. 20, pp. 197–209.
135. Sutherland, J.B., Rafii, F., Khan, A.A., and Cerniglia, C.E., *Microbial Transformation and Degradation of Toxic Organic Chemicals*, Young, L.Y. and Cerniglia, C.E., Eds., New York: Wiley, 1995.
136. Bogan, B.W. and Lamar, R.T., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 5, pp. 1597–1603.
137. Bogan, B.W., Lamar, R.T., and Hammel, K.E., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 5, pp. 1788–1792.
138. Bohmer, S., Messner, K., and Srebotnik, E., *Biochem. Biophys. Res. Commun.*, 1998, vol. 244, no. 1, pp. 233–238.
139. Johannes, C., Majcherczyk, A., and Huttermann, A., *Appl. Microbiol. Biotechnol.*, 1996, vol. 46, no. 3, pp. 313–317.
140. Pickard, M.A., Roman, R., Tinoco, R., and Vǎzquez-Duhalt, R., *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 9, pp. 3805–3809.
141. Collins, P.J., Kotterman, M.J.J., Field, J.A., and Dobson, A.D.W., *Appl. Environ. Microbiol.*, 1996, vol. 62, pp. 4563–4567.
142. Beaudette, L.E., Davies, S., Fedorak, P.M., *et al.*, *Appl. Environ. Microbiol.*, 1998, vol. 64, pp. 2020–2025.
143. Fahr, K., Wetzstein, H.G., Grey, R., and Schlosser, D., *FEMS Microbiol. Lett.*, 1999, vol. 175, no. 1, pp. 127–132.
144. Novotny, C., *Folia Microbiol.* (Prague), 1997, vol. 42, no. 2, pp. 136–140.
145. Ruttimann-Johnson, C. and Lamar, R.T., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 10, pp. 3890–3893.
146. Ricotta, A., *Bull. Environ. Contam. Toxicol.*, 1996, vol. 57, no. 4, pp. 560–567.
147. Masaphy, S., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 10, pp. 3587–3593.
148. Calvo, A.M., *Arch. Microbiol.*, 1998, vol. 171, no. 1, pp. 31–36.
149. Rodriguez, E., Pickard, M.A., and Vǎzquez-Duhalt, R., *Curr. Microbiol.*, 1999, vol. 38, no. 1, pp. 27–32.
150. Mougin, C., Boyer, F.D., Caminade, E., and Rama, R., *J. Agric. Food Chem.*, 2000, vol. 48, no. 10, pp. 4529–4534.
151. Reddy, G.V. and Gold, M.H., *Microbiology*, 2000, vol. 146, no. 2, pp. 405–413.
152. Reddy, G.V. and Gold, M.H., *Biochem. Biophys. Res. Commun.*, 1999, vol. 257, no. 3, pp. 901–905.
153. Brock, B.J. and Gold, M.H., *Arch. Biochem. Biophys.*, 1996, vol. 331, no. 1, pp. 31–40.
154. Stahl, J.D., Rasmussen, S.J., and Aust, S.D., *Arch. Biochem. Biophys.*, 1995, vol. 322, no. 1, pp. 221–227.
155. Asma, D. and Yesilada, O., *Folia Microbiol.* (Prague), 2002, vol. 47, no. 4, pp. 413–416.
156. Pinheiro, R., Belo, I., and Mota, M., *Appl. Microbiol. Biotechnol.*, 2002, vol. 58, no. 6, pp. 842–847.
157. Bending, G.D., Friloux, M., and Walker, A., *FEMS Microbiol. Lett.*, 2002, vol. 212, no. 1, pp. 59–63.
158. Masaphy, S., Henis, Y., and Levanon, D., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 10, pp. 3587–3593.
159. Moen, M.A. and Hammel, K.E., *Appl. Environ. Microbiol.*, 1994, vol. 60, pp. 1956–1961.
160. Bogan, B.W. and Lamar, R.T., *Appl. Environ. Microbiol.*, 1995, vol. 61, no. 7, pp. 2631–2635.
161. Hammel, K.E., *Environ. Health. Perspect*, 1995, vol. 103, no. 5, pp. 41–43.

162. Sack, U., Hofrichter, M., and Fritsche, W., *FEMS Microbiol. Lett.*, 1997, vol. 152, no. 2, pp. 227–234.
163. Wetzstein, H.G., Stadler, M., Tichy, H.-V., *et al.*, *Appl. Environ. Microbiol.*, 1999, vol. 65, no. 4, pp. 1556–1563.
164. Schlosser, D., Fahr, K., Karl, W., and Wetzstein, H.G., *Appl. Environ. Microbiol.*, 2000, vol. 66, no. 6, pp. 2479–2483.
165. Murado, M.A., Tejedor, M.C., and Baluja, G., *Bull. Environ. Contam. Toxicol.*, 1976, vol. 15, pp. 768–774.
166. Nishimura, K., Yamamoto, M., Nakagomi, T., *et al.*, *Appl. Microbiol. Biotechnol.*, 2002, vol. 58, no. 6, pp. 848–852.
167. Hofrichter, M., Bublitz, F., and Fritsche, W.J., *Basic Microbiol.*, 1994, vol. 34, no. 3, pp. 163–172.
168. Perkins, E.J., Gordon, M.P., Caceres, O., and Lurquin, P.F., *J. Bacteriol.*, 1990, vol. 172, no. 5, pp. 2351–2359.
169. Sanyal, D. and Kulshrestha, G., *J. Agric. Food Chem.*, 2002, vol. 50, no. 3, pp. 499–505.
170. Rigot, J. and Matsumura, F., *J. Environ. Sci. Health*, 2002, vol. 37, no. 3, pp. 201–210.
171. Bordjiba, O., Steiman, R., Kadri, M., *et al.*, *J. Environ. Qual.*, 2001, vol. 30, no. 2, pp. 418–426.
172. Mitsevich, E.V., Mitsevich, I.P., Perelygin, V.V., *et al.*, *Prikl. Biokhim. Mikrobiol.*, 2000, vol. 36, no. 6, pp. 672–678.
173. Bollag, J.M., Myers, C.J., and Minard, R.D., *Sci. Total Environ.*, 1992, vol. 123-124, pp. 205–217.
174. Hatcher, P.G., Boriatynski, J.M., Minard, R.D., *et al.*, *Environ. Sci. Technol.*, 1993, vol. 27, pp. 2098–2103.
175. Nanny, M.A., Boriatynski, J.M., Tien, M., and Hatcher, P.G., *Environ. Toxicol. Chem.*, 1996, vol. 15, pp. 1857–1864.
176. Tatsumi, K., Freyer, A., Minard, R.D., and Bollag, J.M., *Soil Biol. Biochem.*, 1994, vol. 26, pp. 735–742.
177. Kang, K.H., Dec, J., Park, H., and Bollag, J.M., *Water Res.*, 2002, vol. 36, no. 19, pp. 4907–4915.
178. Xu, F. and Bhandari, A., *J. Agric. Food Chem.*, 2003, vol. 51, no. 1, pp. 183–188.
179. Koroleva, O.V., Stepanova, E.V., Landesman, E.O., *et al.*, *Prikl. Biokhim. Mikrobiol.*, 2002, vol. 38, no. 4, pp. 413–418.
180. Koroleva, O.V., Stepanova, E.V., Landesman, E.O., *et al.*, *Toxicol. Environ. Chem.*, 2001, vol. 80, no. 3-4, pp. 175–188.
181. Vasil'chenko, L.G., Khromonygina, V.V., Koroleva, O.V., *et al.*, *Prikl. Biokhim. Mikrobiol.*, 2002, vol. 38, no. 5, pp. 534–539.
182. Martin-Laurent, F., Piutti, S., Hallet, S., *et al.*, *Pest. Management Sci.*, 2003, vol. 59, no. 3, pp. 259–268.
183. Ostrofsky, E.B., Robinson, J.B., Traina, S.J., and Tuovinen, O.H., *Soil Biol. Biochem.*, 2002, vol. 34, no. 10, pp. 1449–1459.
184. Entry, J.A., Donnelly, P.K., and Emmingham, W.H., *Appl. Soil. Ecol.*, 1996, vol. 3, no. 1, pp. 85–90.
185. Mougin, C., Laugero, C., Asther, M., *et al.*, *Appl. Environ. Microbiol.*, 1994, vol. 60, no. 2, pp. 705–708.
186. Mori, T. and Kondo, R., *FEMS Microb. Lett.*, 2002, vol. 213, no. 1, pp. 127–131.
187. Mori, T. and Kondo, R., *Appl. Microbiol. Biotechnol.*, 2002, vol. 60, no. 1-2, pp. 200–205.
188. Wittich, R.M., *Appl. Microbiol. Biotech.*, 1998, vol. 49, no. 5, pp. 489–499.
189. Rosenbrock, P., Martens, R., Buscot, F., *et al.*, *Appl. Microbiol. Biotechnol.*, 1997, vol. 48, no. 5, pp. 665–670.
190. Kodama, T., Ding, L.X., Yoshida, M., and Yajima, M., *J. Mol. Catalysis B-Enzymatic*, 2001, vol. 11, no. 4-6, pp. 1073–1078.
191. Benoit, P., Barriuso, E., and Calvet, R., *Chemsphere*, 1998, vol. 37, no. 7, pp. 1271–1282.
192. Levanon, D., *Soil Biol. Biochem.*, 1993, vol. 25, no. 8, pp. 1097–1105.
193. Chan, K.H. and Chu, W., *Chemosphere*, 2003, vol. 51, no. 4, pp. 305–311.
194. Saltmiras, D. and Lemley, A.T., *Water Res.*, 2002, vol. 36, pp. 5113–5119.