product (*10, 12*). This report directly links the HTLV *tat* product to transcriptional *trans*-activation, whereby host cellular genes involved in T-cell proliferation might be modulated in the absence of adjacent *cis*-acting proviral integrations (*7*). The HTLV *tat* product may share functional similarities to the immortalizing proteins of adenovirus and SV40, as well as to the cellular *myc* oncogene product. Like the HTLV *tat* product, these proteins are located in the nucleus (*22*) and can exert regulatory effects on the transcription of viral or cellular genes (*23*). The expression of a biologically active HTLV *tat* protein in eukaryotic cells should allow a direct examination of the ability of this protein to regulate the transcription of host cell genes and to immortalize primary lymphocytes.

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## Oxidation of Persistent Environmental Pollutants by a White Rot Fungus

Abstract. *The white rot fungus* Phanerochaete chrysosporium *degraded DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane], 3,4,3',4'-tetrachlorobiphenyl, 2,4,5,2',-4',5'-hexachlorobiphenyl, 2,3,7,8-tetrachlorodibenzo*-p-*dioxin, lindane (1,2,3,4,5,6-hexachlorocyclohexane), and benzo*[a]*pyrene to carbon dioxide. Model studies, based on the use of DDT, suggest that the ability of* Phanerochaete chrysosporium *to metabolize these compounds is dependent on the extracellular lignin-degrading enzyme system of this fungus.*

Many toxic or carcinogenic organohalides persist in the environment and tend to accumulate in the body fat of animals occupying higher trophic levels (*1*). One reason for the environmental persistence of these compounds is that microorganisms are either unable to degrade them or do so very slowly (*2*).

Lignin is a naturally occurring, highly complex, nonrepeating heteropolymer that provides structural support in woody plants (*3*). Like many synthetic organohalides, lignin is resistant to attack by most microorganisms. Its biodegradation is thought to be the rate-limiting step in the carbon cycle (*3–5*). Microorganisms that are able to metabolize lignin include some species of fungi and a relatively small number of species of bacteria (*5, 6*). Studies of the lignin-degrading system of *Phanerochaete chrysosporium*, a common white rot fungus (*3, 5–8*), have shown that in nitrogen-, carbohydrate-, or sulfur-deficient cultures this fungus secretes a unique $H_2O_2$-dependent extracellular lignin-degrading enzyme system (*8*). Because of its ability to generate carbon-centered free radicals (*9*), this enzyme system is able to catalyze numerous, nonspecific cleavage reactions on the lignin "lattice." The resultant heterogeneous mixture of low molecular weight aromatic compounds may then undergo further modification or ring cleavage and metabolism to $CO_2$ by more conventional enzyme systems. Furthermore, chlorinated lignin-derived by-products of the Kraft pulping process, as well as chlorinated lignin, are also readily degraded by *P. chrysosporium* (*10, 11*). The ability of this fungus to degrade lignin and to metabolize halogenated aromatics suggested to us that

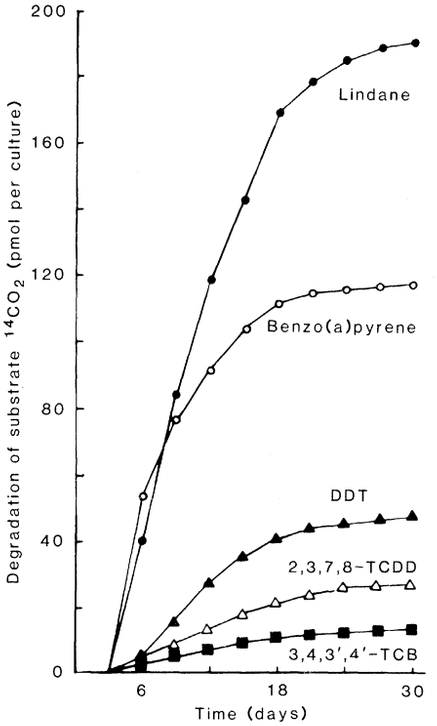


Fig. 1. Rate of oxidation of five environmentally persistent organopollutants to $CO_2$ by *P. chrysosporium* (*13, 17*).

more recalcitrant organohalides, such as DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane] polychlorinated biphenyls, polychlorinated dibenzo(*p*)dioxins and lindane (1,2,3,4,5,6-hexachlorocyclohexane), might also be degraded by this organism. The carbon skeletons of many of these pollutants are found within the structure proposed for the lignin polymer (*3, 12*).

Studies with $^{14}C$-labeled compounds demonstrated that the organopollutants tested [DDT, 2,4,5,2′,4′,5′-hexachlorobiphenyl (2,4,5,2′,4′,5′-HCB), 3,4,3′,4′-tetrachlorobiphenyl (3,4,3′,4′-TCB), 2,3,7,8 - tetrachlorodibenzo - *p* - dioxin (2,3,7,8-TCDD), lindane, and benzo[*a*]pyrene] were oxidized to $^{14}CO_2$ by nitrogen-deficient, ligninolytic cultures of *P. chrysosporium* (*13*) (Fig. 1). Because the radioactive labeling in these compounds was restricted to the ring carbons, we conclude that *P. chrysosporium* is able to degrade halogenated aromatic rings. This is consistent with the results of Leatham *et al.* (*14*), who demonstrated that *P. chrysosporium* contains a constitutive enzyme system capable of cleaving the aromatic ring of 36 aromatic compounds, including 2-chloroisovanillic acid. *Phanerochaete chrysosporium* is also able to dechlorinate alkyl chlorides (it degrades lindane, a nonaromatic compound that is chlorinated on every carbon atom, to $CO_2$).

In cultures (10 ml) containing 1.25 nmol of substrate, 13.8, 27.9, 48.0, 116.8, and 190.8 pmol of 3,4,3′,4′-TCB, 2,3,7,8-TCDD, DDT, benzo[*a*]pyrene, or lindane, respectively, were converted to $^{14}CO_2$ during the 30-day incubation period (Fig. 1). Because of its low radioactivity, the highly chlorinated polychlorobiphenyl congener 2,4,5,2′,4′,5′-HCB was assayed at a higher substrate concentration (5.0 nmol per culture); under these conditions 44.2 pmol per culture were converted to $^{14}CO_2$ during the incubation period. In all cases, $^{14}CO_2$ release first occurred between day 3 and day 6 of incubation and showed a maximal rate between day 3 and day 18, after which $^{14}CO_2$ production continued at decreasing rates until the end of the incubation.

In model studies with [$^{14}C$]DDT, $^{14}CO_2$ release was dependent on the presence of glucose. After the incubation period, the rates of $^{14}CO_2$ production could be increased if glucose was added to the culture. The rate of $^{14}CO_2$ release was dependent on the concentration of glucose in the culture medium. For example, a twofold increase in the glucose concentration (from 56 to 112 m*M*) more than doubled the total amount of $^{14}CO_2$ produced during the incubation period.

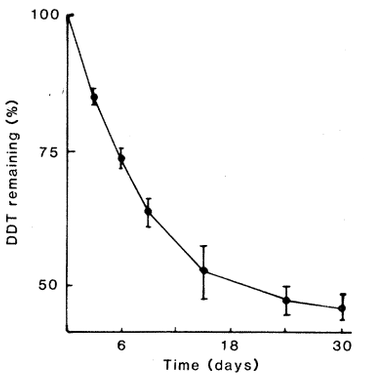


Fig. 2. Rate of disappearance of DDT in cultures of *P. chrysosporium* (*15*).

In other studies, the rate of disappearance of DDT was monitored by gas-liquid chromatography (*15*). Of the DDT initially present in culture, 50 to 60 percent was metabolized during the incubation period (Fig. 2). DDD [2,2-bis(4-chlorophenyl)-1,1-dichloroethane], dicofol [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol], and DBP (4,4′-dichlorobenzophenone) were identified as metabolites (*16*). After the addition of more glucose (56 m*M*) and continued incubation for an additional 18 days, more than 90 percent of the DDT initially present had been metabolized. Furthermore, DDT metabolites were also metabolized.

Lignin degradation in *P. chrysosporium* occurs in nitrogen-deficient cultures after ~3 days of incubation, after which $^{14}CO_2$ release from [$^{14}C$]lignin is observed (*17*). Maximal rates of lignin degradation, as assayed by $^{14}CO_2$ production, were observed between day 3 and day 18 of the incubation. Lignin degradation continued to occur, albeit at successively lower rates, until the glucose was depleted. The same pattern of first release of $^{14}CO_2$ at day 3, maximal activity between days 3 and 18, and subsequent continued metabolism was observed in $^{14}CO_2$ production studies in which ring-labeled [$^{14}C$]DDT was the substrate (Fig. 3). Whereas nitrogen deficiency initiated the degradation of [$^{14}C$]lignin to $^{14}CO_2$, nitrogen-rich cultures suppressed lignin degradation (*17*). The same pattern was observed for the degradation of [$^{14}C$]DDT (Fig. 3).

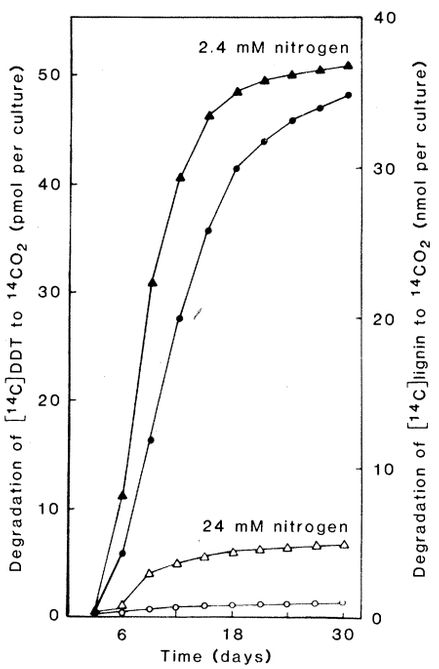


Fig. 3. Effect of nutrient nitrogen concentrations on the oxidation of lignin (triangles) and DDT (circles) to $CO_2$ (*13, 17*).

These results suggest that the same enzyme system may be responsible for the degradation of lignin and organohalides. However, it is possible that two different enzyme systems are synthesized in response to nitrogen starvation.

Earlier studies with microorganisms focused on a number of strategies to demonstrate or enhance the relative abilities of these organisms to degrade various persistent xenobiotics (*18*). Fungi and bacteria are the principal degraders of organic matter. Because organic nutrients are often presented to fungi as large, insoluble macromolecular complexes, these complexes must first be degraded to smaller substituents, which may then be internalized and used as a source of nutrients. This initial degradation is accomplished as a result of the secretion by the fungi of a group of stable extracellular enzymes, which may include proteases, pectinases, lipases, cellulases, and, in the case of *P. chrysosporium*, ligninases (*8, 19*). Because they are physically adsorbed in soils and sediments, many organopollutants are less available for uptake and metabolism by microorganisms. The action of these extracellular fungal enzymes may provide a mechanism by which some organopollutants are made more accessible for biodegradation.

The free-radical mechanism of the lignin-degrading system of *P. chrysosporium* is highly nonspecific and nonstereoselective (*8, 9*). This is precisely the type of mechanism that one would select for site decontamination, because contamination by only one compound is likely to be the exception rather than the rule.

The very low relative concentrations of some organopollutants in the environment may profoundly affect their susceptibility to biodegradation. It has been postulated that the evolution of microorganisms capable of degrading recalcitrant man-made compounds may occur only in ecological niches where the con-

centrations of these organopollutants are high enough to exert selective pressure (*20*). In some cases, microorganisms may have the enzymatic capability to degrade the organopollutant but the organopollutant may not be present in sufficient concentrations to induce the enzymes required for degradation. In either case, pollutants present in low concentrations will not be degraded. In *P. chrysosporium*, the degradation of organohalides and of lignin is initiated by nitrogen starvation rather than by the presence of substrate. Thus large concentrations of organohalides or other recalcitrant pollutants need not be present to induce the enzymes required to initiate biodegradation.

*Phanerochaete chrysosporium* and related fungi (in the class Basidiomycetes there are between 1600 and 1700 species of wood-rotting fungi) are responsible for recycling carbon bound in lignin (*21*). These fungi may be important in the biodegradation of persistent man-made organic compounds in the environment.

Numerous strategies have been used in the aerobic treatment of contaminated waste effluents, sludges, sediments, and landfills. Among these are activated sludge processes, aerated lagoons, aerobic digestion, trickling filters, rotary biological contactors, and aerobic composts (*22*). The effectiveness of these systems is ultimately dependent upon the microorganisms present in the system. Thus it is critical that the most appropriate organisms (those with a demonstrated inherent ability to degrade a wide range of environmental pollutants) be selected. We propose that biotreatment systems inoculated with *P. chrysosporium* and fortified with a suitable carbohydrate source, under nitrogen-limiting conditions, may provide an effective and economical means for the biological detoxification and disposal of hazardous chemical wastes.

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13. Culture conditions and the $^{14}CO_2$ evolution assay were the same as those described (*17*). Rubber stoppers absorb certain volatile organohalides. Therefore, incubation vessels equipped with Teflon liners similar to those described by A. C. Marinucci and R. Bartha [*Appl. Environ. Microbiol.* **38**, 1020 (1979)] were used in these studies. The possible biosynthesis of volatile intermediates was assayed in control incubations in which the $^{14}CO_2$-containing atmosphere of the incubation was bubbled through a gas scrubber containing 10 ml of scintillation cocktail before being bubbled into the $^{14}CO_2$ trap. No volatile intermediates were detected by this procedure. Uninoculated cultures served as controls; volatilization of the parent compounds did not occur under these conditions. The specific activities for the $^{14}$C-labeled organopollutant used in this study were 8.2, 27.0, 146.0, 40.0, 58.5, and 54.0 mCi/mmol for 2,4,5,2′,4′,5′-HCB, 3,4,3′,4′-TCB, 2,3,7,8-TCDD, DDT, benzo[*a*]pyrene, and lindane, respectively. Synthetic [$^{14}$C]lignin [T. K. Kirk *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2515 (1975)] had a specific activity of 613.6 mCi/mmol. Individual data points in Fig. 1 and Fig. 3 represent the mean of four replicate assays. The standard deviation for each data point in Fig. 1 was ± 6.2, ± 7.2, ± 1.8, ± 1.2, and ± 0.7 pmol or less for lindane, benzo[*a*]pyrene, DDT, 2,3,7,8-TCDD, and 3,4,3′,4′-TCB, respectively. The standard deviation in the presence of 24 m*M* nitrogen in Fig. 3 was ± 1.0 nmol or less for lignin and ± 0.7 pmol or less for DDT. In 2.4 m*M* nitrogen, the standard deviation was ± 1.62 nmol or less for lignin and ± 1.8 pmol or less for DDT.
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15. Cultures (10 ml) of *P. chrysosporium* were homogenized in a hand homogenizer (Potter Elvehjem) with 25 ml of acetonitrile. Saturated NaCl (2 ml) and distilled water (60 ml) were then added, and the mixture was extracted into petroleum ether or hexane (100 ml) in a separatory funnel (two 50-ml portions). Uninoculated cultures served as controls. Culture conditions were the same as those described (*17*). The initial concentration of DDT was 4.8 μ*M*. DDT and its metabolites were quantitated on a Varian gas chromatograph (model 3700) equipped with a 3 percent OV-1 column, an electron-capture detector, and a Hewlett-Packard integrator (model 3390 A) (column temperature, 220°C; injector temperature, 280°C; detector temperature, 348°C).
16. DDD and the DBP were identified by gas-liquid chromatography with authentic standards and by mass spectroscopy. Dicofol was identified by treating the unknown compound with acetic anhydride, which formed a compound that chromatographed with authentic dicofol acetate.
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## Rationale for Development of a Synthetic Vaccine Against *Plasmodium falciparum* Malaria

Abstract. *Protective immunity against malaria can be obtained by vaccination with irradiated sporozoites. The protective antigens known as circumsporozoite (CS) proteins, are polypeptides that cover the surface membrane of the parasite. The CS proteins contain species-specific immunodominant epitopes formed by tandem repeated sequences of amino acids. Here it is shown that the dominant epitope of* Plasmodium falciparum *is contained in the synthetic dodecapeptide Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro-Asn-Ala-Asn-Pro or* $(NANP)_3$. *Monoclonal antibodies and most or all polyclonal human antibodies to the sporozoites react with* $(NANP)_3$, *and polyclonal antibodies raised against the synthetic peptide* $(NANP)_3$ *react with the surface of the parasite and neutralize its infectivity. Since* $(NANP)_3$ *repeats are present in CS proteins of* P. falciparum *from many parts of the world, this epitope is a logical target for vaccine development.*

The development of vaccines against malaria is complicated by the fact that the protective antigens are specific for each of the main developmental stages of the parasite, *Plasmodium falciparum*, in the human host. These stages include the sporozoites, which are injected by mosquito bite; the blood stages, which develop in red cells and cause the clinical disease; and the gametocytes, which are infectious for *Anopheles* mosquitoes. An effective vaccine against the sporozoites would be most advantageous because it would block infection in the human and