Biodegradation of γ -hexachlorocyclohexane (Lindane) by a non-white rot fungus *conidiobolus* 03-1-56 isolated from litter

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Abstract Biodegradation of chlorinated pesticide γ -hexachlorocyclohexane (lindane) by a nonwhite rot fungus *Conidiobolus* 03-1-56 is reported for the first time. *Conidiobolus* 03-1-56, a phycomyceteous fungus isolated from litter, completely degraded lindane on the 5th day of incubation in the culture medium, and GC-ECD studies confirmed that lindane removal did not occur *via* adsorption on the fungal biomass. Degradation studies using different medium compositions showed that nitrogen/carbon limiting conditions (stress conditions) and presence of veratryl alcohol, induced the secretion of extracellular oxidative enzymes, which enhanced the rate of lindane biodegradation. Under optimum nutrient-limiting conditions, GC-ECD and GC-MS analysis showed complete absence of any degradation metabolite, indicating that lindane was completely mineralized. Assays for tannic acid utilization and lignin peroxidase showed similar enzymatic profiles between *Conidiobolus* 03-1-56 and standard white rot fungi *Pleurotus ostreatus* 1200 and *Trametes versicolor* 1086. Although *Conidiobolus* 03-1-56 showed a reduced enzyme activity compared to white rot fungi, preliminary evidence indicates that enzymes responsible for lignin degradation by white rots play a key role in lindane degradation by *Conidiobolus* 03-1-56.

Keywords *Conidiobolus* 03-1-56 · Lindane · Non white – rot · Lignin peroxidases

Introduction

The γ -isomer of hexachlorocyclohexane, commonly known as lindane or γ -HCH, is a highly persistent and recalcitrant organochlorine pesticide [1, 2] Although its use is restricted or completely banned in most countries, it continues to pose serious environmental and health concerns due to its toxicity and bioaccumulation arising from its persistence in water, soil and sediments. Adverse health effects associated with lindane and other isomers include neurological problems and immunosuppression in humans and liver cancer in rats and mice [3]. Lindane is relatively recalcitrant under aerobic conditions [2, 1, 4] but aerobic and anaerobic lindane degradation using bacteria has been reported [5, 6, 7, 8, 2, 9 and 10]. However, only a few bacterial species are known to completely mineralize lindane in the laboratory and open environments. Therefore, the search for new bioremedial agents capable of rapid and complete lindane mineralization remains a scientific challenge.

Bioremediation of a wide variety of pollutants such as azo dyes, heavy metals (chromium) and pesticides (DDT and lindane) have been reported using fungi [11]. There are advantages in using fungi for bioremediation, as they possess extra-cellular enzymes and their mycelia provide deeper penetration and large surface area. Fungi can thrive on bulk pollutant matter, thus enhancing the bioavailability of compounds having low water solubilities such as lindane. Additionally, the non-specific nature of

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their enzyme system enables the fungi to degrade complex mixtures of pollutants upto CO₂. This is very important because the metabolites formed during the degradation process cause toxicity, to the same extent or even greater than the parent substance. The degrading system of fungi, however, is usually induced by nutrient depletion and not by a particular pollutant. In this way, the fungi can degrade very low concentrations of a pollutant, without any pre-conditioning [12].

Among fungi, only white rot species (WRF) show high efficiency in degradation of a variety of xenobiotic compounds. *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Trametes hirsutus*, *Coriolus versicolor*, *Daldinia concentrica*, *Cyathus bulleri*, *Phanerochaete sordida* and *Phanerochaete chrysosporium* are few examples of WRF known to degrade pesticides, azo dyes, chemical compounds, explosives, etc. [13, 14, 15, 16, 17, 18 and 19]. The capability of white-rot fungi to degrade pollutants like lindane is based on the production of highly potent, non-specific extracellular enzymes (peroxidases, phenol oxidases, manganese and lignin peroxidase, laccases) that catalyze a wide range of one- and two-electron oxidations, leading to the breakdown of organic compounds [20, 21]. The production and activity of these enzymes are two prerequisites for successful application of white rot fungi in bioremediation. Lindane biodegradation using WRF has been reported using different strains of *Pleurotus* such as *florida*, *sajor-caju*, *eryngii*, *ostreatus*, and by other white rots such as *Cyathus bulleri*, *Phanerochaete chrysosporium* and *Trametes hirsutus* [22, 15, 16 and 23].

Although white rot fungi possess such an efficient enzyme system, they are very slow growing species, and in some cases, also require oxygen-rich environments [24] for their growth. Moreover, the aging of fungal mycelium and the risk of contamination by bacteria under non-sterile conditions have hindered the application of white rot fungi in wastewater treatment [25]. To overcome these disadvantages of white rots, attempts should be made to isolate fast growing non-white rot fungal cultures capable of rapid and complete lindane degradation. The enzymatic profile of such cultures would then play an important role in determining the extent of lindane degradation in the medium.

The present study reports lindane biodegradation using a non-white rot, phycomyceteous fungi *Conidiobolus* 03-1-56 isolated from litter. Under carbon/nitrogen limiting conditions, GC-ECD and GC-MS analysis showed complete absence of degradation metabolites. Enzyme assays showed that *Conidiobolus* 03-1-56 possessed a similar enzyme profile to white rot fungi but with a lower activity.

Experimental procedures

Chemicals

Lindane (γ -HCH, > 99.9 % purity) was procured from Sigma Chemicals, USA. Fungal culture media were supplemented with 5 mg/L of lindane using a stock solution of lindane (1000 mg/L) prepared in acetone (purity, ~ 99 %, Merck Chemicals, UK).

Growth and Maintenance of Fungal Cultures

Conidiobolus 03-1-56 fungal culture was isolated from litter. For comparing the extracellular enzyme activity of *Conidiobolus* 03-1-56 with standard white-rot fungi (WRF), *Pleurotus ostreatus* 1200 and *Trametes versicolor* 1086 were procured from National Chemical Laboratory Culture Collection (NICM), Pune, India. The culture was maintained on malt extract-yeast extract-peptone-glucose (MGYP) medium [26] at 28°C. The fungal culture was grown in liquid medium in 250 mL Erlenmeyer flasks containing 50 mL of medium at 28°C under shaking conditions at 150 r.p.m. WRF were maintained on potato dextrose agar medium.

Degradation of Lindane by *Conidiobolus* 03-1-56

Culture-containing MGYP broth was supplemented with 5 mg/L of lindane in 250 mL Erlenmeyer flasks containing 50 mL of medium at 28°C under shaking conditions at 150 r.p.m. Lindane degradation from culture medium was monitored on 0, 2nd, 5th and th day of incubation. Media without lindane served as controls. Samples of the culture media (1 mL) were pipetted out at fixed time intervals and centrifuged at 8000 g for 10 min to separate the fungal mass from the medium. Residual lindane and its degradation products were estimated using gas chromatography. All experiments were done in triplicates. For Plate Clearance Assay, a loopful of fungal culture was spot inoculated on the medium. Plates were incubated at 28°C for 5 days. Solidified MGYP medium was supplemented with 1000 mg/L lindane. The solubility of lindane in water is only 7.3 mg/L at 25°C. Excess lindane in the solution precipitated and gave a chalky appearance to the medium. A loopful of fungal culture was spot inoculated on the medium. Plates were incubated at 28°C for 5 days.

Lindane Adsorption on Fungal Mycelia

To confirm whether lindane removal from solution was due to degradation or by adsorption onto the fungal mycelia, fungal biomass grown in MGY medium supplemented with lindane (5mg/L), was centrifuged, washed three times in sterile distilled water and then washed with HPLC grade n-hexane to remove adsorbed lindane and its degradation products. The mycelial mats were also homogenized in a mortar and pestle and extracted with hexane. Both n-hexane fractions were analyzed using GC. All experiments were done in triplicates.

Gas-Chromatography

For gas chromatographic determination of the residual lindane in the synthetic medium, 100 μ L supernatants were extracted in 1 mL n-hexane (HPLC grade, Qualigens Chemicals, India), by vortexing for 5 min. Residual lindane was analyzed using a Gas Chromatographic system (Perkin Elmer Auto system XL) with ECD detector using capillary column SGE 3780B21 (length- 25m, type-bonded phase, material- silica, phase-BP5 (non polar), film thickness-0.5 μ m). The conditions of analysis were, oven temperature – 240°C, injector temperature – 230°C and detector temperature – 280°C, carrier gas: nitrogen at 35.0 mL/min. Retention time of lindane for the isothermal method was 2.9 min. Detection of degradation metabolites was carried out using a thermal ramping method: the column temperature was started at 50°C, held for 10 min, ramped up to 150°C at a rate of 10°C/min, held for 10 min, ramped to 240°C at a rate of 10°C/min and finally held for 15 min. The temperature of the injector and detector were maintained at 230°C and 280°C respectively. The retention time for lindane was 15 min in the thermal ramping method.

GC-MS was performed on a Trace GC 2000 (Thermo) model equipped with a Mega 5 MS capillary column (30 m, 0.25 mm ID and 0.5 μ m film thickness). The column temperature started at 50°C, held for 10 min, ramped upto 150°C at a rate of 10°C/min, held for 10 min, ramped to 240°C at a rate of 10°C/min and finally held for 15 min. EI—MS was carried out at 15 eV ionization potential and 230°C ion source temperature. 15 μ L samples were injected each time.

Optimization of the Nutritional Environment of the Fungus

Different sets of media compositions were used (Table 1) to monitor lindane degradation by *Conidiobolus* 03-1-56 under nitrogen/carbon starved conditions.

Plate Clearance Assay for Tannic Acid Utilization (Bavendamm Method)

Modified Bavendamm medium was prepared and autoclaved at 120°C for 15 minutes. Medium contained malt extract 1.5 %, tannic acid 0.05 %, glucose 0.2 % and Bacto-Difco agar 2 %. Plates were prepared and standard white-rot fungi and *Conidiobolus* 03-1-56 were spot inoculated. Plates were incubated for 48 h at 28°C.

Lignin Peroxidase (LiP) Assay

The reaction mixture for ligninase assay contained 50 mM tartaric acid (pH 3.0), 2 mM veratryl alcohol and 500 μ L of diluted cell-free culture fluid in a final volume of 1 mL. The reaction was started by addition of 0.4 mM H₂O₂ solution. The formation of veratraldehyde was monitored at 310 nm. A calibration plot for the above assay was initially calculated using same reaction medium but containing varying concentrations of 0.4 mM H₂O₂ and no culture fluid. The absorbance at 310 nm was monitored at fixed time intervals after the addition of H₂O₂ (plot linearity was observed between 0-3 minutes beyond which there were deviations). The enzyme activity in the culture fluid was calculated in terms of U/L unit (U) was defined as the amount of enzyme that oxidized 1 μ mol of veratryl alcohol in 1 min.

Results and discussion

Biodegradation of lindane by *Conidiobolus* 03-1-56

Gas chromatographic analysis of the fungal growth medium indicated that on the fifth day of incubation, lindane degradation was mediated by *Conidiobolus* 03-1-56, while no removal was observed in the control medium. GC analyses of cell-free extracts from the *Conidiobolus* 03-1-56 culture medium showed the presence of only one peak on the day zero samples, corresponding to lindane. This peak intensity decreased substantially on the third day, with the appearance of an additional peak at a lower retention time. On the fifth day of the experiment, complete degradation of lindane and disappearance of the second peak was observed. The additional peak corresponded to the degradative product of lindane. The results clearly indicated that the utilization of lindane by the fungus started only after the depletion of easily degradable nutrients in the medium. Thus, complete lindane degradation by the fungus was achieved on the fifth day of incubation. Plate clearance

Table 1 MGYP media compositions for lindane degradation by *Conidiobolus* 03-1-56 under nutritional stress conditions

Set	<i>Conidiobolus</i> 03-1-56 grown in MGYP medium containing per 50 mL					
	Glucose	Malt Extract	Peptone Extract	Yeast Extract	Lindane	Veratryl Alcohol
A	0.05 g	0.15 g	0.25 g	0.25 g	5 mg/L	-
B	0.001 g	0.15 g	0.25 g	0.25 g	5 mg/L	-
C	0.001 g	0.15 g	0.025 g	0.025 g	5 mg/L	-
D ^a	0.001 g	0.015 g	0.025 g	0.025 g	5 mg/L	0.4 mM

^aFungus was pre-grown in MGYP medium for 48 h, centrifuged at 8000 r.p.m. and washed 2-3 times with sterile water to remove residual medium impurities. The washed fungal pellet was spot inoculated in fresh MGYP medium.

assay using *Conidiobolus* 03-1-56 was also performed as a parallel test to study the degradation ability of this fungus. After 5 days of incubation, a zone of clearance was observed around the fungus, showing its ability to utilize lindane (Fig. 1) as a carbon source.

GC analysis of the mycelial extract on the fifth day of incubation did not show the presence of lindane. This indicated that lindane removal from the medium was not due to adsorption on the fungal mycelia. Attempts to study lindane degradation by *Conidiobolus* 03-1-56 in basal minimal salts medium supplemented with lindane were not successful due to its inability to grow in the presence of inorganic N-containing nutrient sources. It had an obligatory requirement of organic sources for its growth.

Enhanced biodegradation of lindane by *Conidiobolus* 03-1-56 under nutritional stress

Use of non-white rot fungi, having similar extracellular enzymes as WRF, has not yet been explored for degradation of organic pollutants. In order to develop a potential lindane bioremediation technology using *Conidiobolus* 03-1-56, a fast growing nonwhite rot fungal strain, we carried out optimization of process parameters for enhanced lindane utilization by this fungus. Standardization of culture and nutritional conditions to achieve enhanced lindane degradation was a crucial factor. Drastic changes in the medium conditions (pH, temperature or use of basal minimal medium with only lindane as sole carbon source) led to the death of this fungal culture. Hence, modifications were carried out in the medium constituents, in a stepwise manner, wherein the effect of changes in the culture environment on the fungal morphology was constantly monitored microscopically. Simultaneous GC analysis was also performed for studying lindane degradation under these specific culture conditions.

It is known that extracellular enzymes produced by fungi (particularly WRF) are various isoforms of extracellular

Fig. 1 Detection of lindane degradation by *Conidiobolus* 03-1-56 using the plate clearance assay.

oxidases (laccases, Mn peroxidase, lignin peroxidase) involved in the degradation of lignin [24, 25]. These enzymes are produced during their secondary metabolism, and hence synthesis and secretion of these enzymes is often induced by limited nutrient levels (mostly C or N) [27,28]. These enzymes are also generated when excess amount of toxic pollutants present in the culture media lead to stress conditions. This enzyme secretion under stress contributes to the degradation of various xenobiotic pollutants. Additionally, veratryl alcohol (3,4-dimethoxybenzyl alcohol), a fungal metabolite and a lignin peroxidase substrate, functions as an inducer for such enzymes [29].

Taking into account the above knowledge, enhanced lindane removal by *Conidiobolus* 03-1-56 under nutritional stress, the fungus was grown in four different media compositions (Table 1). It was observed that pre-grown *Conidiobolus* 03-1-56 could degrade ~ 95 % lindane in

Fig. 2 GC-MS spectrum of control sample (5 mg/L lindane) and treated sample (5 mg/L lindane treated with fungus *Conidiobolus* 03-1-56 for 5 days). Control sample shows peak at 24.64 min corresponding to lindane and the mass spectrum associated with the chromatographic peak of lindane. Note that the ordinate units for control sample are $\times 10^7$ and for treated sample are $\times 10^5$.

48 h. Hence, pre-grown fungal biomass was used in all four compositions. GC analysis of set **A** showed complete lindane removal with 72 h. In sets **B** and **C**, lindane degradation was more rapid compared to set **A**, i.e., in 48 h. In set **D**, however, complete lindane degradation occurred in only 24 h. To further confirm this result, GC-MS of set **D** was performed. Figure 2 shows the GC-MS analysis, which indicated complete lindane removal. No peak

corresponding to any degradation metabolite was observed. This indicates that lindane was completely mineralized to CO_2 . Experiments to quantify the mineralization efficiency using ^{14}C lindane are currently in progress.

A 50-fold decrease in carbon source concentration only (set **B**) increased the rate of lindane degradation compared to set **A** (48 h compared to 72 h). A simultaneous 10-fold decrease in nitrogen source concentration (set **C**), however,

Fig. 3 Kinetics of lindane degradation using *Conidiobolus* 03-1-56 under nutritional stress. [lindane] = 5 mg/L

did not change the rate of lindane degradation compared to **set B**. But addition of veratryl alcohol (**set D**) resulted in a rapid increase in the lindane degradation activity of *Conidiobolus* 03-1-56. Data in Figure 3 show that complete removal of lindane from the medium was achieved in 24 h. GC-MS analysis of the corresponding samples confirmed the absence of lindane and other degradation metabolites.

Enhanced lindane degradation in carbon- and nitrogen-limiting conditions along with the addition of veratryl alcohol has been reported for white-rot fungi such as *Pleurotus ostreatus*, *Trametes hirsutus* and *Phanerochaete chrysosporium*. [11, 15 and 30]. In the present study, the enhanced lindane degradation shown by *Conidiobolus* 03-1-56 under stress conditions was caused by the production of LME and LiP. This probable process of enzyme mediated enhancement in degradation finds similarities with mechanistic pathways of pollutant destruction by WRF.

Lindane-degrading enzymes produced by *Conidiobolus* 03-1-56

To determine the type of lindane-degrading extracellular enzymes secreted by *Conidiobolus* 03-1-56, plate clearance assays were performed for lignin-modifying enzymes (LME) and lignin peroxidases (LiP). Figure 4 shows a zone of clearance in plates containing tannic acid and incubated with *Conidiobolus* 03-1-56 for 48 h. The plates also showed a prominent brown–blackish pigmentation (Fig. 4). Presence of LME is confirmed by tannic acid

Fig. 4 Plate clearance assay for tannic acid utilization: *Conidiobolus* 03-1-56 (topleft), *Pleurotus ostreatus* 1200 (top-right) and *Trametes versicolor* 1086 (bottom row showing the reverse side of the same plate for zone of clearance and brown-blackish pigmentation).

utilization, which produces a zone of clearance or zone of oxidation and pigmentation in solid medium. Lignin peroxidase (LiP) catalyzes the oxidation of veratryl alcohol by H_2O_2 to veratraldehyde. The alcohol exhibits no absorbance at 310 nm whereas the aldehyde absorbs strongly at that wavelength (molar extinction coefficient = $\epsilon_{310} = 9333 \text{ M}^{-1} \text{ cm}^{-1}$). This property was used to determine LiP concentration in a continuous spectrophotometric assay [14]. The activity of the enzyme in the culture fluid of *Conidiobolus* 03-1-56 was calculated as 7.7 U/L. Comparison of these enzyme profiles was done with standard white-rot fungi *Pleurotus ostreatus* and *Trametes versicolor*. LiP activities of the standard cultures were also measured as 84.1 U/L and 93.2 U/L respectively. Thus, it was evident from the above preliminary experiments, that *Conidiobolus* 03-1-56 showed an enzymatic profile similar to that of standard white rot fungi, albeit with a reduced activity (7.7 U/L compared to 84.1 or 93.2 U/L).

Conclusion

Conidiobolus 03-1-56, a non-white rot phycomyceteous fungus, completely degraded lindane in culture medium on 5th day of incubation. GC analysis indicated that adsorption of lindane on fungal mycelia was not responsible for lindane removal from medium. Lindane was utilized as a carbon source by *Conidiobolus* 03-1-56 only after all other

carbon sources were exhausted. Enzyme assays showed the presence of lignin modifying enzymes and lignin peroxidase in the culture medium, which were responsible for lindane degradation. Optimization of nutritional environment in culture medium showed that nitrogen/carbon limiting conditions (stress conditions) induced the secretion of these extracellular enzymes, which enhanced the rate of lindane degradation. Secretion of such enzymes under stress conditions finds a parallel with enzyme secretion in white rot fungi. *Conidiobolus* 03-1-56 showed an enzymatic profile similar to that of standard white rot fungi such as *Pleurotus ostreatus* and *Trametes versicolor*, albeit with reduced enzyme activity. We would like to emphasize here that in spite of possessing low enzyme activities, *Conidiobolus* 03-1-56 rapidly degraded lindane (~ 24 h only) under optimum medium composition. This unique behavior exhibited by a non-white rot fungal species is noteworthy and is the first report of lindane degradation by non-white rot fungi. The mechanistic pathway of lindane degradation by this fungus is being elucidated, and further research work to get a critical insight into the mechanism of lindane degradation is being carried out.

References

- Deo PG, Karanth NG, and Karanth NGK (1994) Biodegradation of hexachlorocyclohexane isomers in soil and food environment. *CRC Crit Rev Microbiol* 20:57–78
- Bachman A, Walet P, Wijnen P, Bruin, W, Huntjens, JLM, Roelofsen W *et al* (1988) Biodegradation of α - and β -hexachlorocyclohexane in soil slurry under different redox conditions. *Appl Environ Microbiol* 54:143–149
- Frankenberger WT (2002) Temperature and pH effects on biodegradation of hexachlorocyclohexane isomers in water soil and soil slurry. *J Agric Food Chem* 50:5070–5076
- Johri AK, Dua M, Tuteja D, Saxena R, Saxena DM, and Lal R (1998) Degradation of alpha, beta, gamma and delta-hexachlorocyclohexanes by *Pseudomonas paucimobilis*. *Biotechnol Lett* 20:885–887
- Mecksongsee B and Guthrie FE (1965) Degradation of chlorinated hydrocarbon insecticides by certain bacteria in broth culture, *J Elisha Mitchel Sci Soc* 81:81–89
- Jagnow G, Haider K and Ellwardt PC (1977) Anaerobic dechlorination and degradation of hexachlorocyclohexane isomers by anaerobic and facultative anaerobic bacteria. *Arch Microbiol* 115:285–290
- Heritage A and MacRae I (1977) Identification of intermediates formed during degradation of hexachlorocyclohexanes by *Clostridium sphenoides*. *Appl Environ Microbiol* 33:1295–1297
- Haider (1979) Degradation and metabolization of lindane and other hexachlorocyclohexane isomers by anaerobic and aerobic soil organisms. *Z Naturforsch* 34C:1066–1069
- Boyle AW, Häggblom MM and Young LY (1999) Dehalogenation of Lindane (γ -hexachlorocyclohexane) by anaerobic bacteria from marine sediments and by sulphate-reducing bacteria. *FEMS Microbiol Ecol* 29:379–387
- Gupta A, Kaushik CP and Kaushik A (2000) Degradation of hexachlorocyclohexane (HCH, α , β and δ) by *Bacillus circulans* and *Bacillus brevis* isolated from soil contaminated with HCH. *Soil Biol Biochem* 32:1803–1805
- Shah MM, Barr DP, Chung M and Aust SD (1992) Use of white rot fungi in the degradation of environmental chemicals. *Toxicol Lett* 6465:493–601
- Bumpus JA, Tien M, Wright D and Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. *Science* 228:1434–1436
- Mougin C, Pericaud C, Dubroca J and Asther M (1997) Enhanced mineralization of lindane in soils supplemented with the white rot basidiomycetes *Phanerochaete chrysosporium*. *Soil Biol Biochem* 29:1321–1324
- Arisoy M and Kolankaya N (1997) Biodegradation of Lindane by *Pleurotus sajorajau* and toxic effects of Lindane and its metabolites on mice. *Bull Environ Contam Toxicol* 59:352–359
- Singh BK and Kuhad RC (1999) Biodegradation of lindane (gamma-hexachlorocyclohexane) by the white-rot fungus *Trametes hirsutus*. *Lett Appl Microbiol* 28:238–241
- Singh BK, Kuhad RC, Singh A, Tripathi KK and Ghosh PK (2000) Microbial degradation of the pesticide lindane (gamma-hexachlorocyclohexane). *Adv Appl Microbiol* 47:269–298
- Lee SM, Koo BW, Lee SS, Kim MK, Choi DH, Hong EJ, et al. (2004) Biodegradation of dibutylphthalate by white rot fungi and evaluation of its estrogenic activity. *Enzy Microb Technol* 35:417–423
- Rigas F, Dritsa V, Marchant R, Papadopoulou K, Avramides EJ and Hatzianestis AI (2005) Biodegradation of lindane by *Pleurotus ostreatus* via central composite design. *Environ Int* 31:195–196
- Sanghi R, Dixit A and Guha S (2006) Sequential batch culture studies for the decolorization of reactive dye by *Coriolus versicolor*. *Biores Technol* 97:396–400
- Hattaka A (1994) Lignin modifying enzymes from selected white rot fungi: production and role in lignin degradation. *FEMS Microbiol Rev* 13:125–135
- Gadd GM (2001) *Fungi in Bioremediation*, Cambridge, UK: Cambridge University Press
- Mougin C, Pericaud C, Dubroca J, and Asther M (1996) Biotransformation of the insecticide lindane by the white rot basidiomycetes *Phanerochaete chrysosporium*. *Pest Sci* 47: 51–60
- Tekere M, Ncube I, Read JS and Zvaunya R (2002) Biodegradation of the organochlorine pesticide, lindane by a sub-tropical white-rot fungus in batch and packed bed bioreactor systems. *Environmental Technology* 23: 199–206
- Pointing S (2001) Feasibility of bioremediation by white rot fungi. *Appl Microbiol Biotechnol* 57:20–33
- Borchert M and Judy JL (2001) Decolorization of reactive dyes by the white rot fungus *Trametes versicolor* in sequencing batch reactors. *Biotechnol Bioeng* 75:313–321
- Sastry M, Ahmad A, Khan MI and Kumar R (2003) Biosynthesis of metal nanoparticles using fungi and actinomycete. *Curr Sci* 85:162–170

27. Tien M and Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization and catalytic properties of a unique H₂O₂-requiring oxygenase. Proc Natl Acad Sci USA 81:2280–2284
28. Haglund C (1999) Biodegradation of xenobiotic compounds by the white rot fungus *Trametes trogii*, M.Sc. Dissertation, Uppsala University
29. Faison BD and Kirk TK (1985) Factors involved in the Regulation of a Ligninase Activity in *Phanerochaete chrysosporium*. Appl Environ Microbiol 49: 299–304
30. Collin PJ, Field JA, Teunissen P and Dobson ADW (1997) Stabilization of lignin peroxidases in white rot fungi by tryptophan. Appl Environ Microbiol 63:2543–2548