Note

Degradation of White-Rot Fungi under
Nutrient-Rich Conditions

Arata KATAYAMA,* Satoko UCHIDA
and Shozo KUWATSUKA

Laboratory of Soil Science, School of Agriculture,
Nagoya University, Chikusa-ku,
Nagoya 464-01, Japan

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INTRODUCTION

Of the wood-rotting fungi, a white-rot fungus *Phanerochaete chrysosporium* has been reported to degrade a wide spectrum of persistent organochlorine compounds including DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane).¹⁻³⁾ The degrading capability is considered due to a family of lignin-degrading enzymes, collectively referred to as ligninases. It is of great interest to utilize the capability of lignin-degrading fungi for cleanup of contaminated sites and for degradation of xenobiotics in soil.^{4,5)} However, extensive degradation of xenobiotics occurs only under nutrient-limited conditions, since ligninases are secreted by *P. chrysosporium* during the secondary metabolism triggered by nitrogen starvation.⁶⁾ Ligninases are produced in larger amounts in 100% O₂ culture atmosphere than in the air (21% O₂).⁷⁾ If a white-rot fungus is capable of producing ligninases in nutrient-rich conditions as well as in the air, the fungus is expected to degrade xenobiotics under changeable conditions such as in soil. We screened 41 strains of wood-rotting fungi, of which two white-rot fungi degraded the persistent organochlorine compound DDT extensively under both nitrogen-limited and nitrogen-rich conditions as well as in the air.

MATERIALS AND METHODS

The two fungal isolates used were *Phlebia strigoso-zonata* 001A and an unidentified wood-rotting basidiomycete F7. The former was isolated from rotten wood in the campus of Nagoya

University and the latter from a rotten wood board kept in the laboratory, which was not identified because it did not form the fruiting body. *Phanerochaete chrysosporium* BKM F-1767, a gift from Dr. F. Matsumura at University of California, Davis, was also used for comparison.

¹⁴C-DDT (labeled at the phenyl ring, 19.1 mCi/mmol) was purchased from Amersham Japan (Tokyo) and purified just before use by silicagel TLC.

The fungi were incubated in two liquid culture media: the nitrogen-limited medium was composed of 56 mM of glucose, 0.6 mM of NH₄NO₃, 0.6 mM of L-asparagine, trace minerals and vitamins in 10 mM of 2,2-dimethylsuccinate buffer (pH=4.5),⁷⁾ and the nitrogen-rich medium of the same composition, except for 10-fold NH₄NO₃. A piece of fungal hyphae was inoculated to 10 ml of nutrient solution in a glass petri dish (10 cm in diameter and 5 cm in height) and incubated at 28°C in the dark for 4 days. Then, 3.01 nmol of ¹⁴C-DDT (125,000 dpm) in 5 or 8 µl of ethanol solution was added and incubated for 3 weeks. CO₂ evolved was trapped in 20% KOH solution placed in the petri dish. The KOH solution was changed every 7 days, and the radioactivity was measured by liquid scintillation spectrometry. Volatilized compounds were trapped in polyurethane foam placed in the petri dish. After 3 weeks of incubation, the fungal hyphae were macerated in 30 ml of acetone with a hand homogenizer and extracted with 50 ml of *n*-hexane twice. Radioactivity in the combined hexane extract and the aqueous solution was measured by liquid scintillation spectrometry. The polyurethane foam was extracted with *n*-hexane and the radioactivity was measured in the same manner. The hexane extracts were concentrated and analyzed with a HPTLC (Whatman HPTLC LHP-KDF). The developing solvent was a mixture of *n*-hexane, ether and glacial acetic acid (100:1:1).⁸⁾ Radioactivity on TLC was measured with a beta-ray scanner (AMBIS Radio-analytic Imaging System).

RESULTS AND DISCUSSION

Table 1 shows the distribution of radioactivity after 3 weeks of incubation with ¹⁴C-DDT in white-rot fungal cultures and the recovery of radioactivity. DDT degradation by the fungi was evaluated by disappearance of DDT, by formation of water-soluble compounds and by CO₂

* To whom all correspondence should be addressed.

Table 1 Distribution of radioactivity after 3 weeks of incubation with ^{14}C -DDT in white rot fungal cultures and the recovery of radioactivity.^{a)}

Fungi	Nutrient nitrogen	Distribution of ¹⁴ C radioactivity (%)						Recovered ¹⁴ C radio-activity (%)
		Acetone/hexane extracts			Water soluble	CO ₂	Volatilized ^{b)}	
		DDT	DDE	Others				
<i>P. chrysosporium</i>	Limited	23.9	7.4	14.2	17.1	0.14	30.0	92.7
BKM F-1767	Rich	44.2	8.4	8.1	19.3	0	20.8	100.8
<i>P. strigosozonata</i>	Limited	21.6	9.0	16.4	23.4	0.10	28.5	99.0
001A	Rich	7.2	3.2	7.8	46.6	0.11	36.0	100.9
Unidentified	Limited	17.6	5.2	8.0	48.6	0.14	16.3	95.8
F7	Rich	9.6	1.1	6.7	60.8	0.22	15.9	94.3

^{a)} Values are the mean of triplicate experiments. The initial amount of ^{14}C -DDT was 3.01 nmol in 10 ml culture (100 $\mu\text{g/l}$).

^{b)} Volatilized compounds from the nitrogen-rich culture of *P. chrysosporium* BKM F-1767 and the cultures of *P. strigosozonata* 001A consisted of DDT (88.7%), DDE (10.1%) and other hexane extractable compounds (1.2%). The composition was almost identical to that of autoclaved cultures. Volatilized compounds from the nitrogen-limited culture of *P. chrysosporium* BKM F-1767 and the cultures of unidentified F7, on the other hand, consisted of DDT (83.7%), DDE (11.1%) and other hexane extractable compounds (5.3%).

evolution. The proportions of DDT disappearance in *P. strigosozonata* 001A and unidentified F7 cultures were higher in the nitrogen-rich medium than in the nitrogen-limited medium, while the proportion in *P. chrysosporium* BKM F-1767 culture was higher under nitrogen-limited conditions, as reported before.²⁾ The proportion of DDT disappearance was higher by *P. strigosozonata* 001A and unidentified F7 than by *P. chrysosporium* BKM F-1767.

More water-soluble fractions were formed in *P. strigosozonata* 001A and unidentified F7 cultures than in *P. chrysosporium* BKM F-1767 culture, especially under nitrogen-rich conditions. The proportions of water-soluble fractions in the two fungal isolate cultures were higher under nitrogen-rich conditions, while in the culture of *P. chrysosporium* BKM F-1767, the proportion was not significantly different under the two nutrient conditions.

Less than 1% of ^{14}C -DDT was mineralized. In the cultures of *P. strigosozonata* 001A and unidentified F7, $^{14}\text{CO}_2$ evolved under nitrogen-rich conditions as well as under nitrogen-limited conditions, while *P. chrysosporium* BKM F-1767 did not mineralize DDT under nutrient-rich conditions.

It was evident that the two white-rot fungi, *P. strigosozonata* 001A and unidentified F7, degraded DDT extensively under both nitrogen-limited and -rich conditions in the air. They had stronger degrading capability than *P. chryso-*

sporium BKM F-1767 under the experimental conditions.

In the hexane extracts of *P. strigosozonata* 001A and unidentified F7, two metabolites, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (DDE) and 2,2,2-trichloro-1,1-bis(4-chlorophenyl)ethanol (dicofol), were identified by co-chromatography. DDE was produced even in an autoclaved fungal culture, which suggested the non-biological formation of DDE. The amount of DDE was smaller in intact cultures, which suggested the degradation of DDE. DDE formation has not been observed under 100% O_2 atmospheric conditions.²⁾ Our results indicated that DDE was produced in the air (21% O_2). The identification of dicofol indicates that a pathway *via* dicofol is included in the DDT metabolism by *P. strigosozonata* 001A and unidentified F7, as in the one by *P. chrysosporium* BKM F-1767.²⁾ No 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (DDD) was observed in all the cultures, although *P. chrysosporium* BKM F-1767 has been reported to metabolize DDT to DDD in the early stage of the growth.²⁾ The reason why no DDD was detected in this study might be due to that measurement was done after 3 weeks of incubation.

The two isolates, especially unidentified F7, degraded DDT more extensively than *P. chrysosporium* BKM F-1767 under both nutrient-rich and nutrient-limited conditions as well as in the air. To date, various bacteria and fungi have been reported as DDT degraders, but their de-

gradation ability was not substantial with the exception of *P. chrysosporium*.⁹⁻¹²⁾ Besides, white-rot fungi has been reported to degrade xenobiotics adsorbed to soil as well as humic acids in soil-suspended culture.⁴⁾ These indicate that the two isolates have a higher potentiality for microbial remediation of contaminated soil. This line of research should be warranted.

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REFERENCES

- 1) J. A. Bumpus, M. Tien, D. Wright & S. D. Aust: *Science* **228**, 1434 (1985)
- 2) J. A. Bumpus & S. D. Aust: *Appl. Environ. Microbiol.* **53**, 2001 (1987)
- 3) D. C. Eaton: *Enzyme Microb. Technol.* **7**, 194 (1985)
- 4) K. H. Haider & J. P. Martin: *Soil Biol. Biochem.* **20**, 425 (1988)
- 5) A. Katayama & F. Matsumura: *Environ. Sci. Technol.* **25**, 1329 (1991)
- 6) M. Tien: *CRC Crit. Rev. Microbiol.* **15**, 141 (1987)
- 7) T. K. Kirk, E. Schults, W. J. Connors, L. F. Lorenz & J. G. Zeikus: *Arch. Microbiol.* **117**, 277 (1978)

- 8) J. P. E. Anderson, E. P. Lichtenstein & W. F. Whittingham: *J. Econ. Entomol.* **63**, 1595 (1970)
- 9) J. P. E. Anderson & E. P. Lichtenstein: *Can. J. Microbiol.* **17**, 1291 (1971)
- 10) F. Matsumura & G. M. Boush: *J. Econ. Entomol.* **61**, 610 (1968)
- 11) M. L. Rochkind-Dubinsky, G. S. Sayler & J. W. Blackburn: "Microbiological Decomposition of Chlorinated Aromatic Compounds," Marcel Dekker, Inc., New York and Basel, pp. 153-162, 1987
- 12) R. V. Subba-Rao & M. Alexander: *Appl. Environ. Microbiol.* **49**, 509 (1985)

要 約

富栄養条件下における白色腐朽菌による DDT の分解

片山新太, 内田聡子, 鍛塚昭三

Phanerochaete chrysosporium BKM F-1767 を対照菌株とし ¹⁴C-DDT (芳香環ラベル) を用いて木材腐朽菌 41 株をスクリーニングした結果, 窒素不足条件下だけでなく十分な栄養条件下でも DDT を生分解できる白色腐朽菌 2 株, *Phlebia strigoso-zonata* 001A と未同定担糸菌 F7, が得られた。この 2 菌株は, 大気条件下かつ十分な栄養条件下で, DDT を水溶性物質さらに ¹⁴CO₂ に変換し, その分解能力は *Phanerochaete chrysosporium* BKM F-1767 を上回った。DDE と dicofol が代謝産物として同定された。本 2 菌株の生物的環境浄化への応用の高い可能性が示唆された。