THE FUNGAL TREATMENT OF THE DIOXIN CONTAMINATED SOIL

Kamei I, Watanabe M, Kondo R
Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka, 812-8581, JAPAN

Abstract
To examine the bioremediation potential of *Phlebia brevispora* in dioxin contaminated soil, it was inoculated to the sterilized soil that was contaminated with 1,3,6,8-tetrachlorodibenzo-*p*-dioxin (1,3,6,8-tetraCDD). Although the growth of the fungus was improved with organic-rich soil, the degradation of 1,3,6,8-tetraCDD was strongly inhibited in organic-rich soil. These results indicate that the existence of organic material was the main inhibition factor of bioremediation potential of white-rot fungi in soil. The degradation of 1,3,6,8-tetraCDD was improved under slurry state condition than solid state condition. When the historically contaminated paddy soil was treated by *P. brevispora* under slurry state condition, one of the main contaminants, 1,3,6,8-tetraCDD was degraded 30%.

Introduction
Dioxins is one of the harmful chemicals which has a risk to cause long-term contamination in the soil or bottom sediment, because it has a chemically stable structure and it accumulates in the environment for a long time. Environmental pollutants are a serious concern worldwide because of the hazards they pose to the health of humans and animals. One method that has become increasingly popular for decontamination of the environment has been bioremediation. One of the early reports indicated that lignin-degrading white-rot fungi, as exemplified by *Phanerochaete chrysosporium*, can degrade an extremely diverse group of environmental pollutants. White-rot fungi are wood-degrading basidiomycetes and are among the most active degraders of lignin. It has been proposed that the white-rot fungi have developed unique non-specific enzyme systems with the ability to attack not only lignin but also a broad spectrum of environmental pollutants.
A significant portion of dioxins accumulated in sediments in Japanese lakes and bays was shown to have originated from agrochemicals, especially pentachlorophenol and chloronitrofen (1). In paddy soil, high concentration of OCDD, 1,3,7,9-tetraCDD and 1,3,6,8-tetraCDD were reported (2).
Recently, we reported on several white-rot fungi belonging to the genus *Phlebia* that are capable of hydroxylating and methoxylating PCDDs, 2,7-dichlorodibenzo-*p*-dioxin (2,7-diCDD), 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7-triCDD), 1,2,8,9-tetrachlorodibenzo-*p*-dioxin (1,2,8,9-tetraCDD), 1,2,6,7-tetrachlorodibenzo-*p*-dioxin (1,2,6,7-tetraCDD), and 1,3,6,8-tetrachlorodibenzo-*p*-dioxin (1,3,6,8-tetraCDD) (3, 4). In particular, it was reported that *Phlebia brevispora* TMIC33929 can degrade 1,3,6,8-tetraCDD and that monohydroxy-tetra-CDD, monomethoxy-tetraCDD, dimethoxy-tetraCDD, dimethoxy-triCDD, and 3,5-dichlorocatechol were detected as metabolites from 1,3,6,8-tetraCDD (4). These results indicated that *P. brevispora* could be a good candidate to afford the remediation of paddy-soil contaminated with 1,3,6,8-tetraCDD historically.

Materials and Methods
Soils
In this study, three kinds of soils were selected to compare the influence of various materials, such as some clay minerals and organic substances for the fungal degradation ability. The basic properties of three kinds of soils, Organic-rich soil, organic-poor soil and dioxin contaminated-paddy soil are determined and are shown in Table 1.

Solid state treatment
The granular 'pearlite' was used as the basic material to introduce the fungus mycelium into the soil. The pearlite is constituted mainly from SiO_2, which is the typical inorganic, porous, and water-retentive material. After the test-tubes containing 1 g of pearlite were autoclaved, mycelium and 7 ml of low nitrogen basal III (HCLN) medium was added. The cultures were incubated statically at 25°C for 7 days.
100-ml Erlenmeyer flasks containing 5 g of soil were autoclaved. Then 25 μl of 0.05 mM substrate (1,3,6,8-tetraCDD) *N*,*N*-dimethylformamide (DMF) solution were added and evaporated in the ambient

atmosphere. The mycelium grown on pearlite was added to the flask and was gently stirred. The headspace of each flask was flushed with oxygen, sealed with a glass stopper and sealing tape, and then incubated statically at 25°C.

Table 1 Soil property

	unit	soil		
		Organic-poor	Organic-rich	Paddy-soil
pH		5.4	5.6	5.7
TOC	g/kg	0.7	32.7	3.1
TN	g/kg	0.1	3.1	1.7
Maximum water-holding capacity	g/kg	362	861	550

Slurry state treatment

The fungus was incubated on a potato dextrose agar (PDA) plate at 25°C for 7 days. Then one agar plate with mycelium was transferred to a sterile blender cup containing 50 ml of sterile water and homogenized for 30s. One milliliter of the homogenate was inoculated into a 100 ml and 500 ml flask containing 30 ml and 300 ml of HCLN medium. The cultures were incubated at 25°C for 5 days with a 150-rpm shaking rate (preincubation). 100 ml and 500 ml Erlenmeyer flasks containing 5 g of soil were autoclaved. Then 25 μl of 0.05 mM substrate (1,3,6,8-tetraCDD) *N*,*N*-dimethylformamide (DMF) solution were added and evaporated in the ambient atmosphere. Preincubated cultures were added to the flasks containing contaminated soil and were then incubated 28 days with a 150-rpm shaking rate.

Analytical methods

After incubation, the same amount of 1M KOH-ethanol solution with medium were added to the culture and then incubated at room temperature for 16 h. After addition of 25 μl of 0.05 mM 1,2,8,9-tetraCDD (internal standard), the culture was homogenized and separated into a biomass residue and a fluid fraction. The fluid fraction was extracted three times with *n*-hexane. The residual biomass was air-dried and Soxhlet-extracted with toluene (12 h). The *n*-hexane and toluene extracts were mixed and were washed with concentrated sulfuric acid and with water. The washed layer was dried over anhydrous sodium sulfate and evaporated to a state of dryness. After addition of 25 μl of 0.05 mM 3,3',4,4'-tetrachlorobiphenyl (internal standard) the concentrate was analyzed by gas chromatography/mass spectrometry (GC/MS).

Results and discussion

P. brevispora was inoculated to the solid state culture and the growth was monitored by the increase of the amounts of ergosterol. The amounts of ergosterol in organic-rich soil culture are higher than in organic poor soil culture (data not shown). Therefore, this fungus can grow well under solid-state soil condition. However, when *P. brevispora* was incubated under solid state condition, degradation of 1,3,6,8-tetraCDD was remarkably inhibited though 1,3,6,8-tetraCDD was effectively degraded under liquid culture condition (Fig.1). This result indicate that solid-state condition is not suitable for the degradation of 1,3,6,8-tetraCDD. Then contaminated soils were treated by *P. brevispora* under slurry-state condition including 30 ml HCLN medium. The degradation rate of 1,3,6,8-tetraCDD under organic-poor soil was improved, approximately 70% of initially added 1,3,6,8-tetraCDD was degraded. However, there was no degradation of 1,3,6,8-tetraCDD under organic-rich soil (Fig.2). Paddy-soil which containing 1,3,6,8-tetraCDD, approximately 25% of 1,3,6,8-tetraCDD was degraded. The organic materials in paddy-soil are fewer than that in organic rich soil, and are higher than that in organic-poor soil (Table 1). These results indicate that the existence of organic material was the main inhibition factor of bioremediation potential of white-rot fungi in soil condition. Then contaminated soils were treated by *P. brevispora* under slurry-state condition including 300 ml HCLN medium. The degradation rate of 1,3,6,8-tetraCDD was improved, approximately 80% of additional substrate in both organic-poor and rich soil was degraded (Fig. 3). Finally, slurry-state condition at 300 ml was applied for the treatment of historically contaminated paddy-soil. Contaminated 1,3,6,8-tetraCDD was degraded 30% (Fig. 4). Although degradation

processing efficiency calls for further investigation, our results clearly demonstrate that contaminated 1,3,6,8-tetraCDD in historically contaminated paddy-soil can be degraded by fungal slurry-treatment.

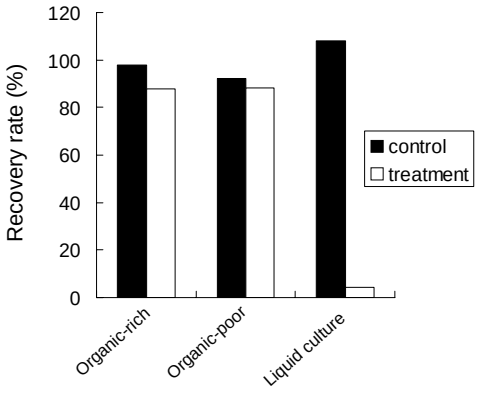

Fig. 1 Degradation of 1,3,6,8-tetraCDD in solid-state soil condition.

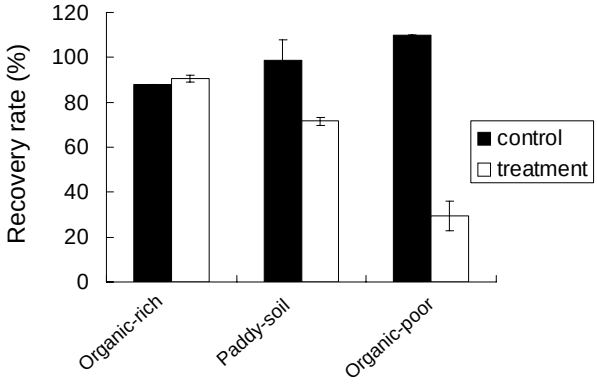

Fig. 2 Degradation of 1,3,6,8-tetraCDD in 30 ml slurry-state soil condition.

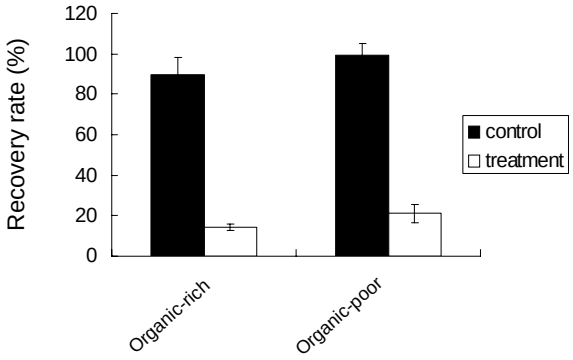

Fig. 3 Degradation of 1,3,6,8-tetraCDD in 300 ml slurry-state soil condition.

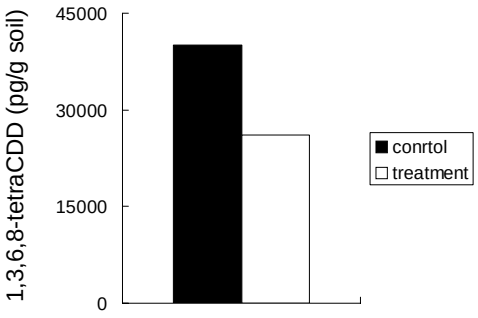

Fig. 4 Degradation of historically contaminated 1,3,6,8-tetraCDD in paddy-soil under 300 ml slurry condition.

Acknowledgements
This work was supported in part by a Grant-in-aid (hazardous chemicals) from the Ministry of Agriculture, Forestry, and Fisheries of Japan (HC-07-2444-1).

References

1. Masunaga S, Takasuga T, Nakanishi J. *Chemosphere* 2001; 44:873.
2. Seike N, Otani S, , Ueji M, Takasuga T, Tsuzuki N. *J Environ Chem* 2003; 13:117.
3. Kamei I, Kondo R. *Appl Microbiol Biotechnol* 2005; 68:560.
4. Kamei I, Suhara H, Kondo R. *Appl Microbiol Biotechnol* 2005; 69:358.