DEGRADATION OF PCDDs/PCDFs BY LIGNINOLYTIC FUNGUS *Phanerochaete sordida* YK-624

Takada, S.[A], Nakamura, M.[A], Matsueda, T.[A], Kurokawa, Y.[A], Fukamati, K.[A], Kondo, R.[B] and Sakai, K.[B]
[A]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano Dazaifu Fukuoka, 818-01, Japan
[B]Faculty of Agriculture, Kyushu University, 6-10-1 Hakozaki Higashi-ku Fukuoka, 812, Japan

Introduction

White rot fungus *Phanerochaete chrysosporium* has shown promise as an organism suitable for the mineralization of a variety of environmentally persistent pollutants, such as polynuclear aromatic hydrocarbons, polychlorinated biphenyls, lindane and DDT. [1] We have isolated several highly ligninolytic white rot fungi from forests, [2] so that embarked on research attempting to apply the fungi to the degradation of environmentally persistent organochlorides. There are several reports on degradation of polychlorinated dibenzo-*p*-dioxins (PCDDs) by the microorganism.[3-7] All of those PCDDs studied, however, are the PCDDs substituted less than four chlorine atoms. The degradation of PCDDs by *P. chrysosporium* has only been carried out on low chlorinated compounds, that is, 2,3,7,8-tetrachloro-[6] and 2,7,-dichlorodibenzo-*p*-dioxin.[7] The present study was undertaken with intent of developing the biodegradation methods of PCDDs andpolychlorinated dibenzofurans (PCDFs). By using the method, degradation of PCDDs and PCDFs by one of the highly ligninolytic fungus isolated was performed.

Materials and Methods

Strain.

Ligninolytic white rot fungus *Phanerochaete sordida* YK-624 strain[2),8)] which was isolated from rotted wood, was used in this study.

Chemicals.

Non-labeled and C^{13}-labeled 2,3,7,8-isomers of tetra-octa PCDDs/PCDFs were obtained from Cambridge Isotope Laboratories (Mass. USA).

Biodegradation methods of PCDDs/PCDFs.

The fungus was grown in low nitrogen medium[9)] (10ml volume) stationarily at 30°C under ambient atmosphere in a 100ml Erlenmeyer flask. After incubation for 7 days, 1ml of 10% glucose was added to each of inoculated flasks and the headspaces were flushed with oxgen; then 10µl of 0.5mg/l stock solution of each 2,3,7,8-substituted PCDDs/PCDFs dissolved in ethyl acetate (each 500 pg) was added, and the flasks were sealed with a glass stopper and a sealing tape. The cultures were incubated for 3, 7, 10 and 14 days (each in duplicate). For 10 and 14 day-incubation samples, glucose was added to the cultures and the flasks were purged with oxgen on day 7. After incubation, 5ml of hexane and internal standards of ^{13}C-PCDDs/PCDFs (each 500 pg) were added to the cultures and then mycelia were dissolved thoroughly with 10ml of concentrated sulfuric acid and the solution was extracted with hexane. The residue in the flask was extracted with acetone and hexane using an ultrasonic generator, and the extract was washed with

water. The combined hexane layers were cleaned up with silica gel chromatography. Uninoculated medium controls were also treated same as mentioned above. Concentrations of substrates were determined by a Varian Model 3400 capillary GC (Quadrex OV-17 column : 0.25μm i. d. X 25m length, 0.1μm film thickness) coupled to a Finnigan MAT-90 MS.

Results and Discussion

Establishment of biodegradation methods.

In the preliminary experiment, we carried out the biodegradation of PCDDs/PCDFs *in* flasks with cotton plugs; however, the loss of PCDDs/PCDFs was observed. The degree of this loss decreased with the number of substituted chlorines. This tendency of the decreases is thought to depend on physicochemical properties, such as solubilities and vapor pressures of PCDDs/PCDFs.[10] Hence, the biodegradation was conducted by sealing with glass stopper after adding glucose and purging with oxygen. A significant difference between the growth of the fungus by this method and that by plugging a flask with cotton was not recognized. After incubation, hyphae were dissolved by adding concentrated sulfuric acid in order to recover quantitatively the PCDDs/PCDFs added. The loss of PCDDs/PCDFs was not observed with this preparation.

Degradation of PCDDs/PCDFs by *P. sordida* YK-624.

Ligninolytic activity of P. sordida YK-624 *was higher than that of P. chrysosporium.*[2] Results of degradation of PCDDs and PCDFs by the fungus YK-624 are shown in Figs. 1 and 2. PCDDs were degraded from 76% (1,2,3,6,7,8-HxCDD) to 48% (1,2,3,4,6,7,8,9-OCDD) by the fungus, comparing with the recoveries (85%-99%) of uninoculated cultures after 14-days incubation (Fig. 1). Similar results were also obtained for PCDFs (Fig. 2). As can be seen in Fig. 1 and 2, the rates of degradation of PCDDs/PCDFs by YK-624 were promoted by adding glucose and purging with oxygen on day 0 and 7. Consequently, it is suggested that the fungus YK-624 could degrade all 2,3,7,8-substituted PCDDs/PCDFs.

Conclusion

By using the biodegradation method developed in this study, the degradation of PCDDs/PCDFs by *P. sordida* YK-624 was observed. To our knowledge, this is the first report on the degradation of highly chlorinated dibenzo-*p*-dioxins (penta- to octa-chloro) and dibenzofurans (tetra- to octa-chloro) by microorganism.

References

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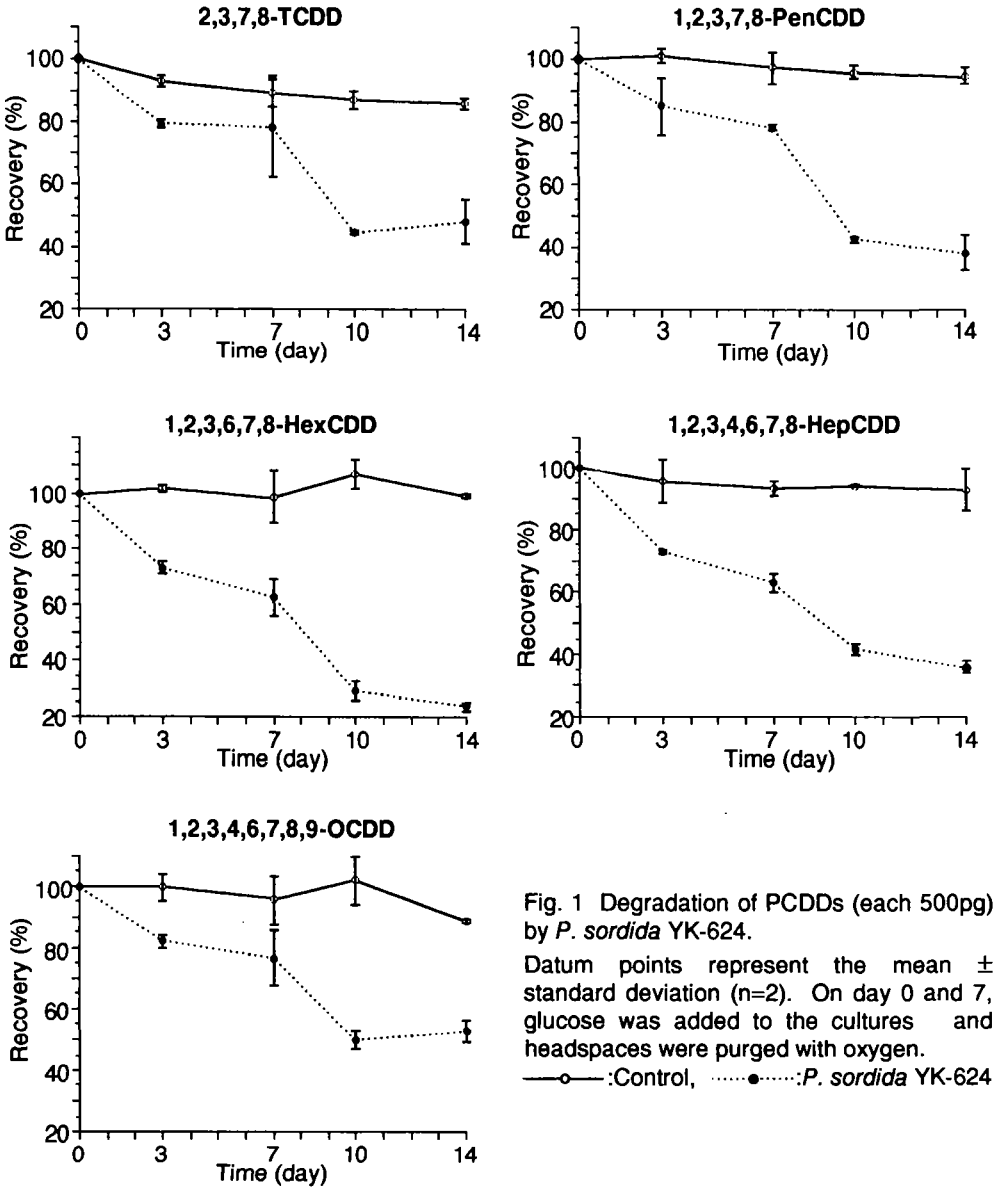

Fig. 1 Degradation of PCDDs (each 500pg) by *P. sordida* YK-624.

Datum points represent the mean ± standard deviation (n=2). On day 0 and 7, glucose was added to the cultures and headspaces were purged with oxygen.

—o—:Control, ·····•·····:*P. sordida* YK-624

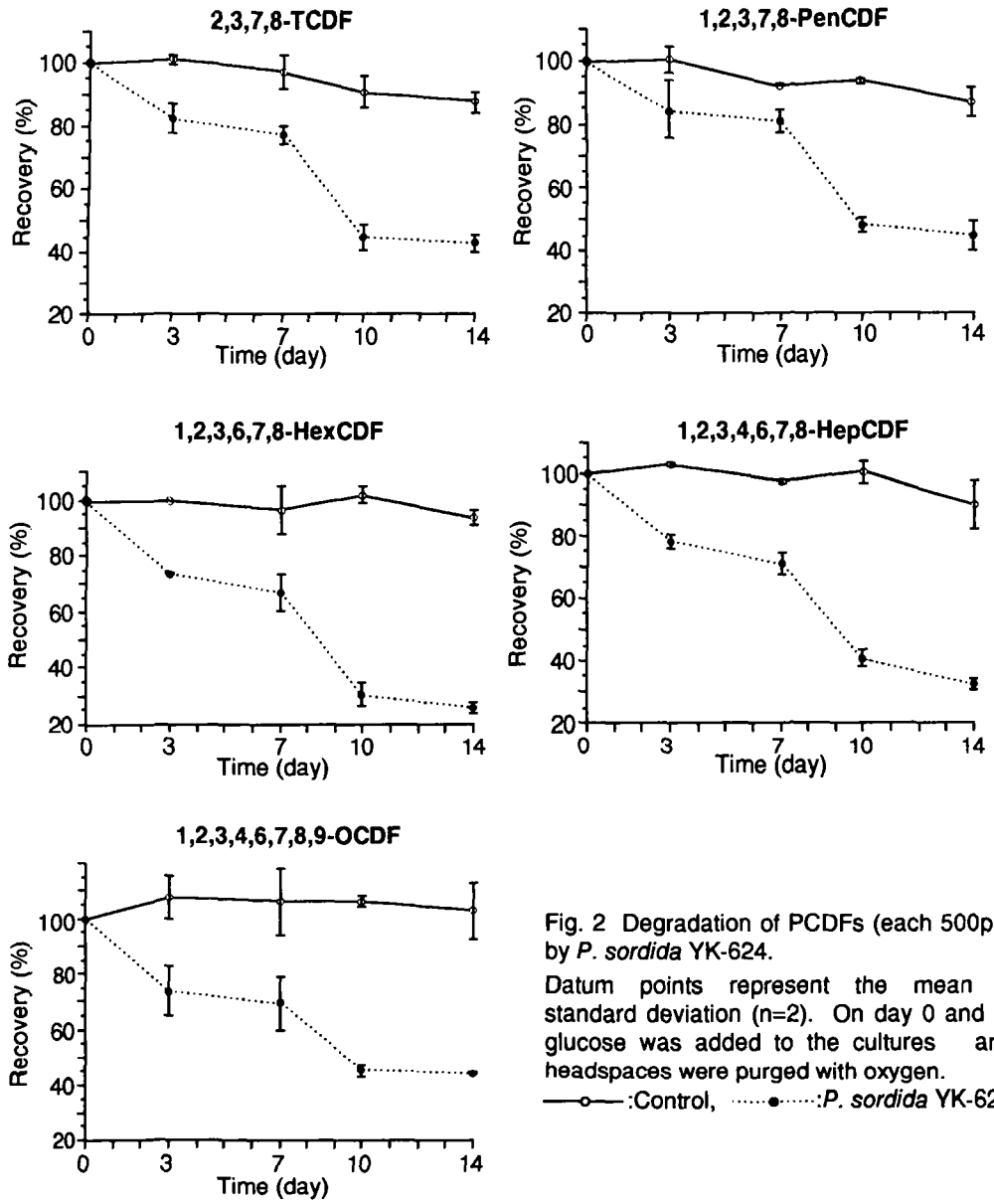

Fig. 2 Degradation of PCDFs (each 500pg) by *P. sordida* YK-624.

Datum points represent the mean ± standard deviation (n=2). On day 0 and 7, glucose was added to the cultures and headspaces were purged with oxygen.

—o— :Control, ·····•····· :*P. sordida* YK-624