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Bioremediation of DDT contaminated soil using brown-rot fungi

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ABSTRACT

The ability of brown-rot fungi (BRF) to eliminate DDT in artificially and historically contaminated soil was investigated to determine whether the BRF would be suitable for the bioremediation of DDT in soil. *Gloeophyllum trabeum*, *Fomitopsis pinicola* and *Daedalea dickinsii* showed an ability to eliminate DDT in artificially contaminated sterilized (SL) and un-sterilized (USL) soils. The addition of Fe^{2+} to the soil system enhanced the ability of some BRF to eliminate DDT. In the contaminated SL soil, the DDT was eliminated by approximately 41%, 9% and 15% by *G. trabeum*, *F. pinicola* and *D. dickinsii*, respectively. Compared with the controls, in the USL soil approximately 43%, 29% and 32% of DDT was eliminated and approximately 20%, 9% and 26% of DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) was detected as a metabolic product with *G. trabeum*, *F. pinicola* and *D. dickinsii*, respectively. Of the BRF, *G. trabeum* demonstrated the greatest ability to eliminate DDT both in the SL and USL soils. *G. trabeum* was applied to a historically contaminated soil which had a DDT concentration more than three times the artificially contaminated soil. *G. trabeum* remediated about 64% of the initial DDT with the addition of Fe^{2+}. There were no significant differences in the results with or without the addition of Fe^{2+}, indicating that *G. trabeum* can be used directly for the degradation of DDT in soil without any other additional treatment. This study identified that *G. trabeum* is the most promising BRF for use in the bioremediation of DDT contaminated soil.

1. Introduction

DDT (1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane) is a persistent pesticide that has been widely used for controlling mosquito-borne malaria and is still used for that purpose in some tropical countries (Quensen et al., 1998; Turusov et al., 2002). DDT was the first synthetic insecticide and became popular all over the world. However, exposure to DDT resulted in increased risk of cancer and endocrine disruption; and the environmental persistence, ecological effects and water insolubility of this insecticide eventually led to a worldwide ban on its use (Turusov et al., 2002). DDT and its metabolites, including DDD (1,1-dichloro-2,2-bis (4-chlorophenyl) ethane) and DDE (1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene) are present and have accumulated at many sites around the world. Therefore, it is important to understand the environmental persistence of this insecticide and to develop effective remediation methods for it.

The removal of DDT from contaminated soils has become an environmental priority, and both physicochemical and biological remediation processes have been studied. Although chemical and physical treatments are more rapid than biological treatments, they are generally destructive and intrusive to affected soils, energy intensive, and may be more expensive than bioremediation (Foght et al., 2001). Considering the bioremediation processes, several bacteria and white-rot fungi (WRF) have been shown to enhance the degradation process in both pure cultures and in soil (Wedemeyer, 1967; Subba-Rao and Alexander, 1985; Bumpus and Aust, 1987; Fernando et al., 1989; Bumpus et al., 1993; Hay and Focht, 2000; Kamanavalli and Ninnekar, 2004). In anaerobic soils, the transformation of DDT to DDD by reductive dechlorination is considered to be the dominant reaction (Boul, 1996), and minor amounts of DDA (bis (4-chlorophenyl) acetic acid), DDM (bis (*p*-chlorophenyl) methane), DDOH (2,2-bis (4-chlorophenyl) ethanol), DBP (4,4-dichlorobenzophenone) and DDE have also been detected (Guenzi and Beard, 1968; Xu et al., 1994; Boul, 1996). In contrast, under aerobic soil conditions DDT is dehydrochlorinated to yield predominantly DDE (Foght et al., 2001).

The ability of brown-rot fungi (BRF) to degrade DDT has been reported previously (Purnomo et al., 2008, 2010c). BRF including *Gloeophyllum trabeum*, *Fomitopsis pinicola* and *Daedalea dickinsii* have shown a good ability to degrade DDT, with the Fenton reaction being an important part of the system (Purnomo et al., 2008, 2010c). Therefore, investigations to determine whether the BRF

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would be able to enhance the degradation of DDT in soil as well as in pure culture were of interest. Thus, the aim of this study is to investigate the ability of BRF to eliminate DDT in an artificially contaminated soil to evaluate the practicality of BRF for remediation applications. As the Fenton reaction enhanced DDT degradation by BRF in pure cultures (Purnomo et al., 2008, 2010c), the effect of the addition of Fe^{2+} as a source for the Fenton reaction in DDT contaminated soil was also investigated. Finally, the best system was applied to a historically contaminated soil.

2. Materials and methods

2.1. Chemicals

DDT and DDD were purchased from the Tokyo Chemical Industry Co. (Tokyo, Japan). Acetone, methanol, *n*-hexane, and *N,N*-dimethylformamide (DMF) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2. Fungi

G. trabeum NBRC6509, *D. dickinsii* NBRC31163 and *F. pinicola* NBRC8705 were obtained from the NITE Biological Resource Center (NBRC; Chiba, Japan). These fungi were maintained as cultures on potato dextrose agar (PDA; Difco, Detroit, MI, USA).

2.3. Culture conditions

Several BRF stock cultures were maintained on 9 cm diameter PDA plates, incubated at 30 °C. To produce cultures for the experiments, mycelia from an agar plate were transferred to a sterile blender cup containing 25 mL sterile water and homogenized for 30 s. A 1 mL aliquot of this homogenate was inoculated onto a 10 mL potato dextrose broth (PDB) medium. The cultures were statically pre-incubated at 30 °C for 5 d.

2.4. Degradation of artificially and historically DDT contaminated soil

An organic-rich soil (Andosol) was purchased from the Japan Plant Protection Association (Ibaraki, Japan). The physicochemical properties of the soil were: pH 5.6; total organic carbon (TOC) content 32.7 g kg^{-1}; total nitrogen (TN) 3.1 g kg^{-1}; total iron (Fe) 5.18 g kg^{-1} and moisture content 26%. The soil texture comprised: sand 43.9% (w/w); silt 40.4% (w/w); and clay 7.8% (w/w). Two grams of soil (dry weight) was autoclaved (121 °C, 20 min), then the sterilized (SL) soil was put into a 100 mL flask, and artificially contaminated with 50 μL of 5 mM DDT in DMF (total amount: 0.25 μmol). The DMF was allowed to evaporate for 3 days at room temperature. Ten milliliters of the pre-incubated fungi in PDB medium were added to the contaminated SL soil, and mixed. The mixture was statically incubated for 14 d at 30 °C. To investigate the contribution of the microbial consortia from the soil, un-sterilized (USL) soil was also used. For a control, the cultures were killed by autoclaving (121 °C, 20 min) before being added to the soil. To investigate the stimulatory effect of the growth medium, USL soil was mixed with PDB medium. The involvement of the Fenton reaction was investigated by the addition of 10 μM of $FeSO_4$ to the system.

A historically contaminated soil was sourced from an industrial DDT contaminated site in Japan, which has an initial DDT concentration of 0.8 μmol g^{-1} soil (moisture content 36%, total iron (Fe) 5.44 g kg^{-1}). Two grams (dry weight) of soil were put into a 100 mL flask. Ten milliliters of pre-incubated fungi in PDB medium were added to the contaminated soil, and mixed. The mixture was statically incubated for 14 d at 30 °C. As a control, the cultures were killed by autoclaving (121 °C, 20 min) before being added to the soil. The involvement of the Fenton reaction was investigated by the addition of 10 μM of $FeSO_4$ to the system.

2.5. Analytical methods

The samples were homogenized with 40 mL of methanol and 120 mL of acetone. After filtration through a 1.0 μm pore glass fiber filter (Advantec, Japan), the filtrates were mixed and evaporated, then extracted with 250 mL of *n*-hexane. The extracts were cleaned up using a Bond Elut Solid Phase Extraction Kit (Varian, USA), evaporated, and then re-dissolved in methanol. The DDT was analyzed by high-performance liquid chromatography (HPLC; Jasco, Japan) with a PU-1500 intelligent pump (Jasco, Japan) and a MD-1510 multi-wavelength detector (Jasco, Japan) fitted with an Inertsil ODS-3 column (150 mm) with an inner diameter of 4.6 mm (GL Science, Japan). The samples were eluted with 82% methanol in a 0.1% trifluoroacetic acid aqueous solution at a flow rate of 1 mL min^{-1} (Purnomo et al., 2010a,b).

2.6. Statistical analysis

The data presented in this paper are the average of three replicates from each experiment. The data were analyzed using the Student's *t*-test to determine any significant differences between or within the groups. Differences between means at a confidence level of 5% ($P < 0.05$) were considered to be statistically significant (Purnomo et al., 2010a,b).

3. Results and discussion

3.1. Degradation of artificially DDT contaminated soil

Soils are a complex environment containing mixed populations of microorganisms with synergistic and antagonistic activities. Soil is not an inert matrix, and its properties and the prevailing environmental conditions will influence the behavior of both microorganisms and DDT. Therefore, it is important to examine DDT degradation in soil, both in the laboratory under controlled conditions, and in the field (Purnomo et al., 2010a,b). In this study, the DDT degradation ability of BRF was investigated in both artificially and historically contaminated soils in the laboratory under controlled conditions. In previous works, *G. trabeum*, *F. pinicola* and *D. dickinsii* all displayed a good ability to degrade DDT in PDB medium pure culture (Purnomo et al., 2008, 2010c). In this study the ability of these fungi to eliminate DDT in soil was investigated to find out whether these fungi are suitable for bioremediation purposes.

In the artificially contaminated sterilized (SL) soil, DDT was eliminated by approximately 36%, 9% and 12% ($P < 0.05$) by *G. trabeum*, *F. pinicola* and *D. dickinsii*, respectively, during the 14 d incubation period (Fig. 1). There was no activity in the control samples, indicating that the inactivated BRF and soil microorganisms did not affect the DDT. In the artificially contaminated un-sterilized (USL) soil control, approximately 41–48% of the DDT was eliminated, indicating soil microorganisms have ability to eliminate DDT which stimulated by the spent fungal cultures. Whereas, approximately 36% of DDT was eliminated by addition of PDB medium in USL soil which stimulated by the growth medium. Since PDB medium contains high levels of nutrients, the addition of PDB medium to soil may improve the microbial population (Chiu et al., 1998; Ribas et al., 2009). The increased soil microbe population may also stimulate microbial metabolism and consequently enhance DDT degradation. Higher DDT removal stimulated by spent fungal cultures indicated that fungal secreted some substances that enhanced the ability of soil microorganisms. Further, in the USL treatment, the DDT was