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Research article

Novel *Chryseobacterium* sp. PYR2 degrades various organochlorine pesticides (OCPs) and achieves enhancing removal and complete degradation of DDT in highly contaminated soil

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ABSTRACT

Long term residues of organochlorine pesticides (OCPs) in soils are of great concerning because they seriously threaten food security and human health. This article focuses on isolation of OCP-degrading strains and their performance in bioremediation of contaminated soil under *ex situ* conditions. A bacterium, *Chryseobacterium* sp. PYR2, capable of degrading various OCPs and utilizing them as a sole carbon and energy source for growth, was isolated from OCP-contaminated soil. In culture experiments, PYR2 degraded 80–98% of hexachlorocyclohexane (HCH) or 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) isomers (50 mg L^{-1}) in 30 days. A pilot-scale *ex situ* bioremediation study of highly OCP-contaminated soil augmented with PYR2 was performed. During the 45-day experimental period, DDT concentration was reduced by 80.3% in PYR2-augmented soils (35.37 mg kg^{-1} to 6.97 mg kg^{-1}) but by only 57.6% in control soils. Seven DDT degradation intermediates (metabolites) were detected and identified in PYR2-augmented soils: five by GC/MS: 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE), 1-chloro-2,2-bis (4-chlorophenyl) ethylene (DDMU), 1-chloro-2,2-bis (4-chlorophenyl) ethane (DDMS), and dichlorobenzophenone (DBP); and two by LC/MS: 4-chlorobenzoic acid (PCBA) and 4-chlorophenylacetic acid (PCPA). Levels of metabolites were fairly stable in control soils but varied greatly with time in PYR2-augmented soils. Levels of DDD, DDMU, and DDE in PYR2-augmented soils increased from day 0 to day 30 and then decreased by day 45. A DDT biodegradation pathway is proposed based on our identification of DDT metabolites in PYR2-augmented systems. PYR2 will be useful in future studies of OCP biodegradation and in bioremediation of OCP-contaminated soils.

1. Introduction

Organochlorine pesticides (OCPs) have been widely applied during the past few decades as insecticides for crop protection and for control of vector-borne diseases such as typhus and malaria. Such OCPs as hexachlorocyclohexane (HCH) and 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) are ubiquitous anthropogenic environmental contaminants (Alonso-Hernandez et al., 2014; Benimeli et al., 2003; Sarkar et al., 2008; van den Berg, 2009) that have been banned in most developed countries for over 30 years. However, because of their long half-life (4–35 yr), they are still abundant in soils in many developed and developing countries worldwide (Corona-Cruz et al., 1999; Cutright and Erdem, 2012; You et al., 1996). DDT, the first synthetic insecticide, was widely marketed and applied in the 1950s and 1960s. Its use was prohibited in most countries starting in the early 1970s because of its toxicity, hydrophobicity, and bioaccumulation (Bajaj et al., 2014; Kannan et al., 1992; Kelce et al., 1995; Purnomo et al., 2011). DDT is an organic compound with low solubility and high chemical stability, composed of chlorinated aliphatic and aromatic structures. It tends to accumulate in food sources and adipose tissues because of its strong lipophilicity (Bidlan and Manonmani, 2002; Chikunia et al., 2002; Dale et al., 1965). Remediation of DDT-contaminated soils is a long-standing, high-priority goal in many countries, and the subject of many research studies.

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Both physicochemical and biological approaches for removal of DDT from soils have been investigated. For examples, the physical approaches of bioventing and thermal treatment and chemical remediation of leaching with surfactants, oxidation, dechlorination by metallic reduction and light degradation (Zhao and Yi, 2010). Remediation based on physicochemical approaches is more rapid than bioremediation; however, it is more expensive and may affect soil physicochemical properties and can cause secondary pollution. Bioremediation, including phytoremediation (Lunney et al. 2003) and microbial bioremediation (Sudharshan et al., 2012), has been applied to remove OCPs from contaminated soils, especially the later one has been proved to be a safe and effective technique for pesticide removal (Foght et al., 2001).

Several microorganisms have been shown to degrade OCPs. *Xanthomonas* sp. ICH12 completely removed 100 mg L^{-1} γ-HCH in liquid culture after 8 days incubation (Manickam et al., 2007). More impressively, *Arthrobacter citreus* BI-100 completely removed this concentration of γ-HCH in 8 h (Datta et al., 2000). Bacterial strains capable of degrading DDT include *Trichoderma viride* (Matsumura and Boush, 1968), *Ralstonia eutrophus* A5 (formerly *Alcaligenes eutrophus*) (Nadeau et al., 1998), and *Pseudomonas acidovorans* (Hay and Focht, 1998). *Eubacterium limosum* ATCC 8486 almost completely removed 100 μM (35.4 mg L^{-1}) DDT in 20 days (Yim et al., 2008). *Pseudoxanthomonas* sp. WAX degraded DDT in co-metabolism with another carbon source in MSM, removing 10% of 20 mg L^{-1} DDT in 72 h (Wang et al., 2010). Most DDT-degrading strains can only degrade DDT in co-metabolism with other carbon sources; they are not able to utilize DDT as a sole carbon and energy source.

There are two metabolic pathways for DDT degradation in bacteria, anaerobic and aerobic degradation pathways. Anaerobic reductive dechlorination (RD) appears to be the predominant mechanism for microbial transformation of DDT (Xiao et al., 2011). DDT was subsequently transformed to such compounds as 1,1-dichloro-2,2-bis (4-chlorophenyl) ethane (DDD), 1,1-dichloro-2,2-bis (4-chlorophenyl) ethylene (DDE), 1-chloro-2,2-bis (4-chlorophenyl) ethylene (DDMU), 1-chloro-2,2-bis (4-chlorophenyl) ethane (DDMS), and finally the dead-end product dichlorobenzophenone (DBP) (Baczynski et al., 2010; Corona-Cruz et al., 1999; Fang et al., 2010; Huang et al., 2007; Lal and Saxena, 1982; You et al., 1996). The first aerobic bacterial degradation of DDT was reported for *A. eutrophus* A5 which could transform both o,p′- and p,p′-DDT with the formation of a yellow product after 30 days of incubation, then further degrade to PCBA (Nadeau et al., 1994). In addition, certain bacteria have been found to metabolize DDT by hydroxylation of the aromatic ring via oxidative attack (Gao et al., 2011; Kamanavalli and Ninnekar, 2004; Nadeau et al., 1994; Sudharshan et al., 2012; Xiao et al., 2011). However, the detail aerobic degradation pathway and intermediates were still remained unknown.

In the present study, a novel bacterium that displays high degrading ability against a wide spectrum of OCPs was isolated and identified as *Chryseobacterium* sp. PYR2. PYR2 is able to use HCH isomers or DDT isomers as a sole carbon and energy source. We investigated its bioaugmentation capability in highly contaminated soil. Microbial degradation of OCPs under *ex situ* conditions is affected by many factors that are not considered in laboratory studies of degrading ability in pure cultures. The objectives of our study were to (1) characterize the ability of PYR2 to degrade OCPs in mineral salt medium, (2) evaluate the potential application of PYR2 for *ex situ* bioremediation of OCP-contaminated soil, and (3) elucidate the metabolic pathway of DDT biodegradation in a PYR2-augmented soil system.

2. Materials and methods

2.1. Chemicals and culture media

Standards of HCH (mixture of α-HCH, β-HCH, γ-HCH, and σ-HCH isomers; purity >99.0%) and DDT (mixture of o,p′-DDT and p,p′-DDT isomers; purity >99.5%), DDE (purity >98.5%), DDD (purity >99.5%), and 1-chloro-2,2-bis (4-chlorophenyl) ethylene (DDMU) (purity >99.0%) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). PCBA and 4-chlorophenylacetic acid (PCPA) were purchased from Sigma–Aldrich, St. Louis, MO, USA. Acetone and acetonitrile (HPLC purity grade) were purchased from Fisher Scientific (Fair Lawn, New Jersey, USA). All other chemicals were of analytical grade and purchased from Beijing Chemical Reagent Co. (Beijing, China). Standard samples were dissolved in acetone as stock solutions (10^5 mg L^{-1}), sterilized by membrane filtration, and added to sterile flasks. Following complete vaporization of acetone, medium was added to obtain the desired sample concentrations.

Enrichment medium (TYC) and mineral salt medium (MSM) were used for culture and isolation of OCP-degrading bacterial strains (Zhang et al., 2010). Luria–Bertani (LB) agar plates and LB liquid medium, both prepared in our lab, were used for strain purification and cultivation respectively, and stored at −80 °C with 15% (w/v) glycerol.

2.2. Contaminated soil

Contaminated soil (continuously contaminated by pesticide chemicals for over 50 years) was collected from the abandoned Anqing Pesticide Plant site in Anhui, China. Multiple samples were collected from 10 cm below the soil surface by quincunx sampling method (Paloma, 2001) and sifted (2 mm mesh size) to remove the cinders and stones and blended thoroughly to give one contaminated soil. Properties of the soil sample are summarized in Table 1. Soil water content was determined by placing samples in an oven at 105 °C for 24 h. Total phosphorus (TP), total nitrogen (TN), total organic carbon (TOC), and texture were assessed previously (Sun et al., 2012).

2.3. Enrichment, isolation, and screening of OCP-degrading bacterial strains

Enrichment and isolation of degrading strains were carried out according to Xu et al. (2008) with some modifications. A homogenized soil sample (10 g) was mixed thoroughly with 100 mL sterilized MSM. A 1-mL aliquot of soil supernatant was transferred into a 50-mL Erlenmeyer flask containing 10 mL MSM and 50 mg L^{-1} OCP (HCH or DDT), and incubated 5 days at 30 °C with rotary shaking (160 rpm). Each culture was subjected to five enrichment transfers (1:10, v/v), appropriately diluted, spread on MSM plates

Table 1
Properties of contaminated soil samples from Anqing Pesticide Plant, Anhui, China.

Property	Value
TOC (g/kg)	7.18
Water content (%)	18.5 ± 1.4
pH	6.5 ± 0.5
Sand (%)	10.53 ± 1.2
Silt (%)	75.12 ± 0.8
Clay (%)	14.35 ± 1.2
Total DDT (mg kg^{-1})	35.4 ± 2.4
Total nitrogen (mg kg^{-1})	18.2
Total phosphorus (mg kg^{-1})	20.6