International Biodeterioration & Biodegradation 132 (2018) 59–65

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biodegradation of organophosphorus insecticides with P—S bonds by two *Sphingobium* sp. strains

Jae-Hyung Ahn, Shin-Ae Lee, Soo-Jin Kim, Jaehong You, Byeong-Hak Han, Hang-Yeon Weon, Se-Weon Lee*

Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration (RDA), Wanju-gun, Jeollabuk-do, 55365, Republic of Korea

ARTICLE INFO

Keywords:
Organophosphorus
Cadusafos
Biodegradation
Soil
Bacteria

ABSTRACT

Although cadusafos, an aliphatic organophosphorus (OP) insecticide, is not approved by the European commission, it is used in several countries and sometimes detected as a residue in soils and agricultural products. In this study, two cadusafos-degrading *Sphingobium* sp. strains, K22212 and Cam5-1, were isolated and characterized for use as detoxifying agents of the insecticide. Both strains degraded 100 mg L^{-1} of cadusafos in mineral medium within 12 h through a common metabolite, which was supposed to be dimerized thiophosphates based on its molecular weight. Degradation of cadusafos increased cell growth for Cam5-1 but not for K22212. K2212 and Cam5-1 degraded cadusafos in soil (15 mg kg^{-1} dry soil) within 5 and 2 days, respectively. Both strains also degraded ethoprophos, phenthoate and phorate but not chlorpyrifos and diazinon, indicating that they are specialized for degradation of OP insecticides with at least one single bond connecting phosphorus and sulfur atoms (P—S bond). For both strains, the degradation rate was the largest for ethoprophos, followed by cadusafos, phenthoate, and phorate. Our results indicate that these bacterial strains are effective degraders of OP insecticides with P—S bonds, and in particular, Cam5-1 is more promising for removal of the OP insecticides in soils.

1. Introduction

At present organophosphorus (OP) insecticides are the largest class of insecticides, occupying nearly 44% shares of the global insecticide market in 2016 (Marketsandmarkets, 2017), with more than 100 OP insecticides commercialized.

Due to their widespread use, OP insecticides are sometimes detected in agricultural products (Bai et al., 2006; Kang et al., 2015). In most cases they were reported to be below the maximum residue level (MRL), the maximum concentration of a pesticide residue permitted in or on food commodities and animal feed (GEMS/Food, 1997), but considering the possibility that long-term low-level exposure of some OP insecticides are involved in developmental toxicity (Costa et al., 2013), endocrine disruption (Aguilar-Garduño et al., 2013), affective disorders (Stallones and Beseler, 2016), cancer (Lerro et al., 2015), and hypospadias (Michalakis et al., 2014), continuous monitoring of OP insecticide levels in food and humans is necessary. In addition, OP insecticides have been detected in diverse environments (Abdel-Halim et al., 2006; Fadaei et al., 2012; Kawahara et al., 2005; Kumari et al., 2008) and several studies have showed the toxic effects of OP insecticides on ecosystems (Díaz-Resendiz et al., 2015; John and Shaike, 2015).

Microbial degradation of OP insecticides has been extensively studied as an insecticide-detoxifying tool. The release of the X group (Fig. S1a) by hydrolysis of the single bond between phosphorus and oxygen (P—O bond) or phosphorus and sulfur (P—S bond) has been considered the most significant step of detoxification (Havens and Rase, 1991; Lai et al., 1995; Singh and Walker, 2006). Many bacterial and fungal strains were reported to degrade various OP compounds, and their hydrolysis enzymes were identified and characterized (Iyer et al., 2013; Singh and Walker, 2006; Theriot and Grunden, 2011). The known bacterial organophosphorus hydrolases have no or much lower activity on P—S bonds than on P—O bonds in OP insecticides (Alvarenga et al., 2015; Horne et al., 2002; Iyer et al., 2013; Lai et al., 1995) while two fungal enzymes were shown to effectively cleave P—S bonds (Liu et al., 2001, 2004).

Among OP insecticides, cadusafos is an aliphatic organophosphorus insecticide with two P—S bonds (Fig. S1b) that targets soil-born insects and nematodes. The World Health Organization has classified cadusafos as a Class Ib (highly hazardous) toxin based on the LD_{50} for the rat

* Corresponding author.
E-mail address: leeseweon@korea.kr (S.-W. Lee).

https://doi.org/10.1016/j.ibiod.2018.05.006
Received 22 January 2018; Received in revised form 11 May 2018; Accepted 11 May 2018

(World Health Organization, 2010) and it was also reported to be ecotoxic towards earthworms and soil microbial communities (Fouché et al., 2017). By the European commission, cadusafos has not been approved as a plant protection product because it did not meet the safety requirements (https://ec.europa.eu/food/plant/pesticides_en); however, it is still being used by several other countries, including South Korea. Although cadusafos is reported to have a relatively short soil half-life of 38 days (PPDB, https://sitem.herts.ac.uk/aeru/ppdb/en/), it is sometimes detected in agricultural products and soil samples in South Korea (government investigation); thus, efficient and safe methods to effectively degrade the insecticide are required. In this study, we isolated two bacterial strains that rapidly degrade cadusafos from agricultural soils and characterized their physiology and degradation abilities to potentially use them as cadusafos-detoxifying agents.

2. Materials and methods

2.1. Isolation and identification of cadusafos-degrading microorganisms

Forty-one agricultural soil samples were collected throughout South Korea in 2016 and information of the sampling sites is indicated in Table S1. Soils were sampled from around the crop roots, transferred to a laboratory in an ice box, and preserved at 4 °C until use. Two enrichment procedures for isolating cadusafos-degrading bacteria were used; for enrichment procedure 1, 1 g of soil sample was transferred to 5 mL mineral medium containing 100 mg L^{-1} of a technical product of cadusafos (92.2%), which was kindly donated by NongHyup Chemical, and incubated in a shaking incubator (150 rpm) at 28 °C. Composition of the mineral medium is indicated in Table S2. When a complete degradation of cadusafos was observed, 10% of the culture medium was transferred to fresh medium and the same procedure was repeated two more times. Finally, some of the culture medium was spread on R2A agar medium and incubated at 28 °C. Bacterial strains with distinct colony morphologies were pure-cultured using R2A agar and examined for degradation of cadusafos. In enrichment procedure 2, 20 g of each soil sample were mixed together and a portion of 400 g was sampled from the mixture, to which 10 mL acetone containing 10 mg of a technical product of cadusafos was added (the final cadusafos concentration was 25 mg kg^{-1} soil). Treated soil was dried at room temperature for 3 h to evaporate acetone completely and then incubated in a glass cylinder (diameter, 11 cm; height, 11 cm) at room temperature. The soil mixture was mixed with a spatula every day and the concentration of cadusafos was monitored periodically. When a complete degradation of cadusafos was observed, enrichment procedure 1 was performed.

Isolated bacterial strains were identified by sequencing 16S rRNA genes and their phylogenetic relationships with nearest type strains were inferred by constructing a maximum-likelihood tree using the EzBioCloud (Yoon et al., 2017) and the MEGA program (Tamura et al., 2013).

2.2. Degradation of cadusafos in a mineral medium

Cadusafos-degrading bacteria were cultured on R2A agar medium at 28 °C for 3 days and then the colonies were suspended in a 0.85% NaCl solution until the turbidity reached 1.65 McFarland units, determined by a DEN-1B densitometer (Biosan). The suspension (0.5 mL) was used to inoculate 5 mL mineral medium containing 100 mg L^{-1} of a technical product of cadusafos. Test tubes containing inoculated media were incubated in a shaking incubator (150 rpm) at 28 °C, and cadusafos concentration and cell density were estimated periodically using high-performance liquid chromatography (HPLC) and a DEN-1B densitometer, respectively. At the start and end of the experiments, viable cells were counted by the dilution plate count technique using R2A agar medium. A non-inoculated medium was used as a control and each treatment was performed in duplicate.

2.3. Physiological and biochemical characterization

In addition to R2A agar, growth of cadusafos-degrading strains on trypticase soy agar (TSA; Difco), nutrient agar (NA; Difco), and Luria-Bertani (LB) agar (Difco) was tested. Growth in the presence of sodium chloride (0–3.0% at intervals of 0.5%) and at various temperatures (8–50 °C at intervals of 5–7 °C) was investigated using R2A broth medium. The pH for optimal growth was tested in R2A broth with a pH range of 3.0–12.0 in increments of 1.0 unit. In all growth experiments, the absorbance at 600 nm of the culture medium in a 96-well plate was measured using a microplate reader (SpectraMax 340; Molecular Devices) to determine growth. Other biochemical characteristics were determined using the API 20NE system (bioMérieux) according to manufacturer's instructions.

2.4. Identification of metabolic intermediates

To identify metabolic intermediates of cadusafos degradation, liquid chromatography-high resolution mass spectrometry (LC-HRMS) and gas chromatography-mass spectrometry (GC-MS) with Purge & Trap were performed. A mineral medium containing 100 mg L^{-1} of a technical product of cadusafos was inoculated with strain Cam5-1, incubated at 28 °C with shaking for 18 h and filtered through a syringe filter with 0.2 μm Supor® membrane (Pall) for the analyses.

LC-HRMS analysis was performed on an ACQUITY UPLC System coupled with a SYNAPT G2-*Si* Mass Spectrometer with an ACQUITY UPLC BEH C18 column (2.1 mm × 100 mm, Waters). A mixture of acetonitrile and water (70:30, v/v) was used as the mobile phase at a flow rate of 0.5 mL min^{-1}. The injection volume was 5 μL. The mass range was 50–1200 Da with a resolution of 20,000. Cone voltage was 30 V and trap collision energy was 6 V.

The automated Purge & Trap Sampler JTD-505III (Japan Analytical Industry) was used under the following conditions: desorption temperature, 280 °C; desorption time, 30 min; desorption gas flow rate, 50 mL min^{-1}; cold-trap for sample trapping, −40 °C; for pyrolysis, 280 °C; transfer-line temperature, 280 °C; needle heater, 280 °C; cold-trap heater, 200 °C; head press, 86 MPa; column flow, 1.0 mL min^{-1}; split ratio, 1/100. GC-MS analysis was performed using a GC-MS QP 2010 plus (Shimadzu) under the following conditions: DB-624 column (30 m × 0.251 mm × 1.40 mm, Agilent Technologies); 30–600 mass scan; 0–35 min oven temperature program (40 °C for 3 min hold, 10 mL min^{-1} up to 260 °C, 5 min hold); ion source, 200 °C; transfer line, 250 °C; EM voltage, 20 eV. Identification of chromatographic peaks was performed based on NIST and WILEY libraries.

2.5. Degradation of cadusafos in soil

Upland soil was collected from the experimental upland field of the National Institute of Agricultural Sciences, Wanju, Korea (35°49′29.9″N, 127°02′41.1″E), dried in a greenhouse for two days, and sifted through a 2-mm pore sieve. The soil sample (300 g) was treated with 4.5 mg of a technical product of cadusafos dissolved in 10 mL of acetone (15 mg cadusafos kg^{-1} dry soil) and dried for 3 h to completely evaporate acetone. After treated soil was transferred to a glass cylinder (diameter, 11 cm; height, 11 cm), 10 mL of 0.85% NaCl solution containing 3.2×10^8 CFU of strain K22212 or 2.4×10^8 CFU of strain Cam5-1 (corresponding to 3.2×10^6 CFU of K22212 and 8.1×10^5 CFU of Cam5-1 g^{-1} dry soil) and 30 mL of distilled water were added. Control soil was treated with 10 mL of 0.85% NaCl solution and 30 mL of distilled water. All cylinders were covered with aluminum foil and incubated at 28 °C. Soil pH (1:10) was 6.4 and water content, which was determined by drying at 105 °C for 4 h, ranged between 16 and 17%. Each treatment (control, K22212 treatment, or Cam5-1 treatment) was performed in triplicate and temporal variation of cadusafos