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Characterization of cell-free extracts from fenpropathrin-degrading strain *Bacillus cereus* ZH-3 and its potential for bioremediation of pyrethroid-contaminated soils

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HIGHLIGHTS

- Fenpropathrin was rapidly degraded by cell-free extracts from *Bacillus cereus* ZH-3.
- The cell-free extracts exhibited great activity under variable conditions.
- The cell-free extracts detoxified fenpropathrin to yield simple products.
- Enzymatic extracts significantly enhanced the removal of pesticide residues in soils.
- Using extracts provides new insights into the utilization of degrading microbes.

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ABSTRACT

Synthetic pyrethroid fenpropathrin has received increasing attention because of its environmental contamination and toxic effects on non-target organisms including human beings. Here we report the degradation characteristics of cell-free extracts from fenpropathrin-degrading strain *Bacillus cereus* ZH-3 and its potential for pyrethroid bioremediation in soils. 50 $mg \cdot L^{-1}$ of fenpropathrin was decreased to 20.6 $mg \cdot L^{-1}$ by the enzymatic extracts (869.4 $mg \cdot L^{-1}$) within 30 min. Kinetic constants K_m and V_m were determined to be 1006.7 $nmol \cdot L^{-1}$ and 56.8 $nmol \cdot min^{-1}$, respectively. Degradation products were identified as 3-phenoxybenzaldehyde, α-hydroxy-3-phenoxy-benzeneacetonitrile and phenol by gas chromatography–mass spectrometry (GC–MS). In addition to degradation of fenpropathrin, the cell-free extracts could degrade other pyrethroids including beta-cypermethrin, cyfluthrin, deltamethrin and cypermethrin. Additionally, the reaction conditions were optimized. In the sterile and non-sterile soils, 50 $mg \cdot kg^{-1}$ of fenpropathrin was reduced to 15.3 and 13.9 $mg \cdot L^{-1}$ in 1 d, respectively. Sprayed 100 and 300 $mg \cdot kg^{-1}$ of fenpropathrin emulsifiable concentrate (EC), up to 84.6% and 92.1% of soil fenpropathrin were removed from soils within 7 d, respectively. Taken together, our results depict the biodegradation characteristics of cell-free extracts from *B. cereus* ZH-3, highlight its promising potential in bioremediation of pyrethroid-contaminated soils and also provide new insights into the utilization of degrading microbes.

1. Introduction

Synthetic pyrethroids (SPs) have been widely used in agriculture due to their potent toxic activity against various insect pests, contributing to more than 25% of world insecticide sales (Chen et al., 2013b; Weston et al., 2009). Due to its high specificity to insect pests and low mammalian toxicity, SPs have become the ideal substitutes of organophosphates (OPs) on crops, homes, gardens and disease control (Miyamoto, 1976; Elliott and Janes, 1978). As one of the most popular SPs, fenpropathrin is the first of light-stable pyrethroid insecticide to be synthesized, and its insecticidal activity has been enhanced for the affinity to voltage-gated membrane channels by the addition of a cyano group at the benzylic carbon (Soderlund, 2010). Generally, fenpropathrin is employed as a broad-spectrum insecticide, extensively targeting on various species of mites and insects on fruit, vines, vegetables, cotton, field crops, and glasshouse crops (Solomon et al., 2001; Kwon et al., 2010). However, intensive use of fenpropathrin in agriculture arises insecticidal exposure from urban, agricultural, or mixed-use sources, which has posed a potential threat to human health and environment safety (Schummer et al., 2012). The action mode of fenpropathrin makes it highly and acutely toxic to aquatic organisms as well as non-target insects (Ding et al., 2011; Pakyari and Enkegaard,

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2013). In addition, accumulating evidences demonstrate that fenpropathrin may cause neuro-behavioral toxicity on mammals by chronic exposure and bioaccumulation (Wolansky et al., 2006; Nieradko-Iwanicka and Borzęcki, 2010). Therefore, effective strategies for eliminating fenpropathrin contaminants are needed to guarantee human health and environmental safety.

Bioremediation of organic contaminants is regarded as a safe, efficient and cost-effective approach using active microorganisms or their enzymes to detoxify and degrade pollutants (Cycoń et al., 2011; Arora et al., 2012). Nowadays, biodegradation of pyrethroid contaminants has been a hot issue and numerous researches showed that various species of microorganisms were able to efficiently eliminate pyrethroid residues (Chen et al., 2012, 2013a; Meyer et al., 2013). Most bioremediation cases were realized by the direct contact between wide type microbes and target contaminants (Sharma et al., 2012; Zhang et al., 2011). Obviously, those methods, featured by their simple procedures and reliable results, were widely accepted and imitated, which had contributed to the most isolation of a great number of active strains so far. However, the traditional methods started to reveal drawbacks. Those procedures were time consuming and required a large quantity of microbes. Moreover, difficulties existed in the study of degradation mechanism while unstable experimental results occurred for microbes being easily influenced by multiple environmental factors, which largely limited the practical utilization of active strains. Recently, research findings demonstrated that the key to microbial metabolism of xenobiotic compounds is the active protein or enzymes of applied microorganisms, which significantly accelerated the metabolic reaction while shortening the experimental period (Fan et al., 2012; Guo et al., 2009; Tallur et al., 2008; Zhai et al., 2012). Hence, utilization of active protein or enzymes could provide new insights and helpful solutions in actual bioremediation of contaminants.

Previously, *Bacillus cereus* ZH-3 was isolated from a pesticide-manufacturing wastewater treatment system using an enrichment culture technique (Huang et al., 2010). In this study, we aimed to characterize the cell-free extracts from strain ZH-3, to determine the enzymatic metabolic products of fenpropathrin, and to investigate its degradation potential in contaminated soils. Our findings demonstrated that the cell-free extracts of strain ZH-3 hold great promise for bioremediation of pyrethroid-contaminated environment.

2. Materials and methods

2.1. Chemicals

Fenpropathrin (93%), cypermethrin (94%), beta-cypermethrin (97%), deltamethrin (98%), cyfluthrin (90%), fenvalerate (90%) and bifenthrin (98%) were obtained from Jiangsu Yangnong Chemical Group Co., Ltd, China. The chemicals were dissolved in acetone at a stock concentration of 10 $g \cdot L^{-1}$, and stored in dark bottles at 4 °C for further use. 20% of fenpropathrin emulsifiable concentrate (EC) was supplied from Sumitomo Chemical, Japan. The chromatographic grade acetonitrile was purchased from Sigma-Aldrich, USA. All the other chemicals and solvents used were at analytical grade.

2.2. Microorganism and preparation of the cell-free extracts

Strain ZH-3 was isolated from activated sludge by the method of an enrichment culture, and the bacterium was identified as *B. cereus*. Mineral salt medium (MSM) was supplemented with fenpropathrin as the sole carbon source at a final concentration of 50 $mg \cdot L^{-1}$ before inoculation (Chen et al., 2013a). The inoculum was cultured in 250-mL Erlenmeyer flakes (pH 8.0) and placed to a platform shaker at 30 °C and 160 $r \cdot min^{-1}$.

Cells were harvested by centrifugation at 8000 $r \cdot min^{-1}$ for 15 min, washed with phosphate buffer solution (PBS) (0.2 $mol \cdot L^{-1}$, pH 7.0), and stored at −20 °C until further use. The frozen cells were thawed and resuspended in PBS (0.2 $mol \cdot L^{-1}$, pH 7.0). After an ultrasonic disruption for 10 min (400 W, each cycle by working for 6 s and pausing for 9 s), the crude extracts were centrifuged at 10,000 $r \cdot min^{-1}$ (10 min) and the supernatant was used for further experiments.

2.3. Enzyme assay and kinetic parameters

The reaction mixtures contained of 2.5 mL preheated PBS (0.02 $mol \cdot L^{-1}$, pH 7.0, 50 $mg \cdot L^{-1}$ fenpropathrin, 30 °C) and 0.5 mL preheated cell-free extracts (30 °C). Immersed in constant temperature (30 °C) water-bath for 30 min, the reaction was ended by 0.2 mL trichloroacetic acid (0.5 mol/L). Samples were triplicated and the residual concentrations were determined by high performance liquid chromatography (HPLC) (Chen et al., 2014). A standard curve of protein concentration was obtained by Bradford method and the amount of protein in cell-free extracts was determined.

Kinetic parameters (Michaelis–Menten constant, K_m, and maximal reaction velocity, V_{max}) were determined by linear regression from double-reciprocal plots according to Lineweaver–Burk. Fenpropathrin concentrations were adjusted from 10 to 120 $mg \cdot L^{-1}$. Each sample was reacted with 0.02 $mol \cdot L^{-1}$ of PBS under the optimal conditions and the degradation of fenpropathrin was monitored by HPLC. The algorithm is applied as shown in Eq. (1).

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max}[S]} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}} \quad (1)$$

where v is the reaction velocity, $[S]$ is the initial concentration of enzyme, V_{max} is maximal reaction velocity, and K_m is Michaelis–Menten constant.

2.4. Identification of biodegradation metabolites of fenpropathrin

The metabolic products of fenpropathrin by cell-free extracts were identified by gas chromatography–mass spectrometry (GC–MS) (Agilent 6890N/5975, USA). The metabolites were obtained sequentially with 20, 20 and 10 mL methylene dichloride from the cell-free extracts after acidification with 5 $mol \cdot L^{-1}$ of HCl. The organic layer was dehydrated, dried and re-dissolved in acetonitrile. After filtration with 0.45-μm membrane (Millipore, USA), the samples were subjected to GC–MS. The metabolites identified by mass spectrometry analysis were matched with authentic standard compounds from the National Institute of Standards and Technology (NIST, USA) library database (Chen et al., 2014).

2.5. Potential of various SPs' biodegradation

To determine their ability of degrading various SPs, 0.5 mL of cell-free extracts was inoculated into 2.5 mL preheated PBS (0.02 $mol \cdot L^{-1}$, pH 7.0) supplemented with 50 $mg \cdot L^{-1}$ of fenpropathrin, cypermethrin, beta-cypermethrin, deltamethrin, cyfluthrin, fenvalerate or bifenthrin. Samples without cell-free extracts were set as negative controls. HPLC was employed to determine the degradation performance of extracts on various SPs.

2.6. Effects of degrading conditions on enzyme activity

Using the single-factor experiments, the effects of pH (4.0–10.0), temperature (20–70 °C), reaction time (10–240 min), protein amount (10–50 μL extract solution) and initial concentrations of fenpropathrin (25–500 $mg \cdot L^{-1}$) on enzyme activity were investigated and the optimal conditions were determined. Cell-free extracts were reacted in 0.02 $mol \cdot L^{-1}$ PBS. Each experiment was carried out in triplicate in a constant temperature water-bath, and was finished by the addition of 0.2 mL of trichloroacetic acid (0.5 $mol \cdot L^{-1}$). The controls