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Biodegradation potential of deltamethrin by the *Bacillus cereus* strain Y1 in both culture and contaminated soil

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ABSTRACT

Pesticide residues in soil are closely related to food safety. Microbial degradation is one of the effective ways to remove the pesticide residues. We isolated a potentially deltamethrin degrading bacterium *Bacillus cereus* strain Y1 and characterized its dissipation capability in both culture and contaminated soil. In liquid medium, the optimal temperature, pH value, and inoculum of dissipation were 30 °C, 7.0, and 7.0% (v/v), respectively. The dissipation rates were 99.4% and 22.8% in 96 h when the initial concentration of deltamethrin were 10 and 100 mg l^{-1}, respectively. Dissipation of deltamethrin followed the pesticide degradation kinetic equation at initial concentrations between 5 and 100 mg l^{-1}. Soil sample was contaminated by deltamethrin with a concentration of 10 mg kg^{-1}, inoculation of 10^{10} CFU g^{-1} dry soil, and cultured at 30 °C. The dissipation rate of deltamethrin was 74.9% in 25 days and was only 45.1% in control lacking strain Y1. Results in greenhouse and open field experiments demonstrate that strain Y1 increases the dissipation rate of deltamethrin in both soil and Chinese cabbage. This study provides scientific evidence and support for the agricultural applications of *B. cereus* strain Y1 in bioremediation to reduce pesticide residues.

1. Introduction

Pyrethroid insecticides are artificially synthesized by mimicking the natural pyrethrins (Miguel and Eugenio, 2002; Laffin et al., 2010). The applications and studies of these insecticides started since the determination of their molecular and chemical structures in 1950s (Katsuda, 1999; Hu et al., 2002). These insecticides have unique molecular structures and have been used widely in cotton, tea, fruit, and many types of vegetables, because they are safe to use, highly efficient, environmentally friendly, and are effective against a wide spectrum of pests. In addition to the pests on crops, the pyrethroid insecticides, e.g., deltamethrin, permethrin, cypermethrin, and fenpropathrin, also effectively control the mosquitos and cockroaches (Hasibur et al., 2006).

However, the long-term use of such pesticides has caused many environmental concerns. The residues of these insecticides and their degraded products remain in the environment and harm the environment and human health (George and Kalyanasundaram, 1994; Wijngaarden et al., 2006). Experiments show that pyrethroid insecticides are highly toxic to not only fish and some agriculturally beneficial insects, e.g., bees and silkworms, but also to humans and other mammals (Dorman and Beasley, 1991; Shafer et al., 2005). For example, cypermethrin is potentially a carcinogen (Shukla et al., 2002), while other pyrethroid insecticides affect the functions of estrogen (Kasat et al., 2002). Furthermore, the pyrethroid insecticides are toxic to the mammalian immune system (Grosman and Diel, 2005) and cardiovascular system (Tan et al., 2005), while the insecticides containing α-cyano and halogen elements are highly acutely toxic to fish (Wang et al., 2000). Therefore, the environmental problems caused by this class of pesticides are increasingly drawing attention worldwide (Chi et al., 2007; Hladik

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and Kuivila, 2012; Ensminger et al., 2013).

Deltamethrin is a type of pyrethroid insecticide commonly used to control the agricultural and human health pests due to its broad-spectrum of pest control, low toxicity to mammals, low cost, and highly efficient insecticidal activity (Bolognesi and Morasso, 2000; Grant and Betts, 2004). Although it does not cause mutations in humans, deltamethrin is highly toxic to many aquatic organisms (Bolognesi and Morasso, 2000; Grant and Betts, 2004). Due to the repeated use of this pesticide in the aquatic environments, target animals have shown increased drug resistance. With the increased dosage, the amount of residues is also growing in the fishery environments and making a negative impact on the fishery production. Therefore, understanding the degradation and removal of deltamethrin in the environment is essential in agriculture (Liu et al., 2010; Tang et al., 2014).

Studies show that the chemical degradation and biodegradation are two common ways to remove the residues of the pyrethroid insecticides from the contaminated environments (Li et al., 2010). The chemical degradation includes photolysis and hydrolysis. The photolysis process requires light as an energy source and mainly occurs in the atmosphere or the soil surface. Because pyrethroid insecticides are insoluble in water, they are less prone to hydrolysis. Biodegradation, especially the microbial degradation of organic pollutants, is one of the most important environmental processes to reduce the environmental load of applied pesticides (Hashem and El-Mohandes, 1999; Grant and Betts, 2003; Preeti et al., 2008; Hong et al., 2010). Because microbial degradation uses microbes inhabiting the contaminated soil, these microbes will not generate secondary pollution. Microbial degradation by introducing microbes is more efficient than the degradation of the pesticide under natural conditions. This technology has been applied in many areas due to its simple and convenient operations, fast breeding, and effective ecological restoration. These features of biodegradation make its wide applications in agricultural bioremediation. In the present, many kinds of microorganisms have been isolated to degrade or convert pesticides, confirming and demonstrating that microbial degradation is one of the best ways to control environmental pollution and is showing great prospects (Benimeli et al., 2008; Fang et al., 2008, 2009; Xu et al., 2009; Zhang et al., 2010; Abo-Amer, 2011; Zhang et al. 2012).

Studies show that a variety of bacteria (e.g., actinomycetes), fungi, and algae are capable of degrading or transforming one type of pyrethroid insecticides, cypermethrin (Guo et al., 2009; Qin et al., 2010; Chen et al., 2011; Gant et al., 2002; Hu et al., 2014). However, the studies of microorganisms degrading deltamethrin are still rare (Liu et al., 2010; Tang et al., 2014). In order to effectively control the environmental pollution caused by deltamethrin, we used an enrichment method to isolate a potentially deltamethrin degrading bacterium *Bacillus cereus* strain Y1 from soil contaminated by deltamethrin. We further explore the bioremediation and characterize the dissipation capability of this strain under various environmental conditions, e.g., temperatures, pH values, initial concentrations, and induction, thus providing theoretical basis to biodegradation of deltamethrin using strain Y1 in production.

2. Materials and methods

2.1. Medium and reagents

Deltamethrin (purity of 99.6%) was purchased from the China National Standard Material Center (Beijing, China); hexane, methanol, dichloromethane, and other chemical reagents were of analytical grade. Two types of bacterial culture media were used. Mineral salt medium (g l^{-1}) contained 1.5 g sucrose, 0.1 g KH_2PO_4, 1.00 g $MgSO_4 \cdot 7H_2O$, 0.01 g K_2HPO_4, and 0.1 g NaCl, dissolved in 1000 ml distilled water. Luria–Bertani (LB) liquid medium (g l^{-1}) was composed of 10 g NaCl, 5 g yeast extract, and 10 g peptone, dissolved in 1000 ml distilled water.

2.2. Soil samples

The soil samples were collected at the Agricultural Experimental Station, Jilin Agricultural University, where the deltamethrin was never used. The soil sample (g kg^{-1} dry weight) contained organic matter 28, total nitrogen 1.57, and total phosphorus 1.03, and had a pH value of 6.4. Samples were taken from the top layer (0–15 cm), air-dried at room temperature, mixed thoroughly, and sieved to 2 mm in size, then stored in the dark at 4 °C in sealed polyethylene bags for further use (Ma et al., 2009).

2.3. Microorganisms and preparation of inoculum

Strain Y1 was isolated from soil contaminated by deltamethrin (Zhang et al., 2014). Briefly, deltamethrin was added to soil, which was not previously treated with deltamethrin. Homology analysis of 16S rRNA using the Basic Local Alignment Search Tool (BLAST) at National Center for Biotechnology Information (NCBI) identified strain Y1 (accession number KC247316) as *B. cereus* and currently stored at the Jilin Agricultural University.

The bacterial suspension was prepared in 100-ml flasks containing 20 ml LB liquid medium, incubated with strain Y1 and deltamethrin (10 mg l^{-1}), and^{-1} cultured in the dark at 30 °C and shaked at 150 rpm. When bacterial growth reached logarithmic phase, the culture was then incubated at 4 °C and centrifuged for 5 min at 12,000 rpm. Bacterial cells were washed twice with phosphate buffer (50 mM, pH 7.0) and the optical density (OD_{600}) of the bacterial suspension was measured by using a spectrophotometer (Shimadzu UV-2410) for the following experiments. OD_{600}, measured at a wavelength of 600 nm, is a commonly used index of evaluating the bacterial growth.

2.4. Measurement of dissipation rate of deltamethrin and the growth rate of strain Y1

Bacteria (diluted 1:20) were seeded into a mineral salt medium (100 ml) containing deltamethrin (10 mg l^{-1}) and cultured at 30 °C and 150 rpm. The dissipation rates of deltamethrin were measured in terms of the concentrations of deltamethrin and values of OD_{600} after culturing for 0, 12, 24, 36, 48, 60, 72, 84, and 96 h, respectively.

2.5. Effects of environmental factors on the dissipation of deltamethrin in liquid culture

2.5.1. Initial concentrations of deltamethrin

In the 100-ml flasks containing the mineral salt medium (20 ml, pH 7.0), the concentrations of deltamethrin were adjusted to 5, 10, 20, 50, 70, and 100 mg l^{-1}, respectively, and seeded with strain Y1 (diluted 1:20), incubated at 30 °C and shaked at 150 rpm in the dark. The concentrations of deltamethrin were measured after culturing for 24, 48, 72, and 96 h, respectively. Each experiment was repeated three times and the controls were the same as treatments but without bacteria.

2.5.2. pH value

Bacteria (diluted 1:20) were seeded into the 100-ml flasks continaing mineral salt medium (100 ml) and deltamethrin (10 mg l^{-1}). The pH values of the medium were adjusted to a series of 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, and 9.0. Under 30 °C and 150 rpm, the concentrations of deltamethrin were measured after culturing for 24, 48, 72, and 96 h, respectively. Each experiment was repeated