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Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity

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ABSTRACT

Serratia spp. strain JC1 and JCN13, isolated from activated sludge, could degrade and utilize beta-cypermethrin (beta-CP) as the sole carbon and energy sources for growth in the minimal salt media (MSM). The two strains could effectively degrade beta-CP with total inocula biomass 0.1–0.3 g dry wt L^{-1}, at 20–38 °C, pH 6–9, initial beta-CP 25–1000 mg L^{-1} and metabolize it by cleavage of ester and diphenyl ether to yield 3-phenoxybenzoic acid (3-PBA) and phenol, then completely mineralize it. Response surface methodology (RSM) was used to optimize degradation conditions. Under their own optimal degradation conditions, strain JC1 could degrade 92% beta-CP within 10 days and the degradation rate of strain JCN13 reached 89% within 4 days. Cell surface hydrophobicity (CSH) and biodegradation assays indicated that JCN13 has higher hydrophobicity and degradation ability than JC1, and it means the high hydrophobicity of strains could enhance the degradation of beta-CP.

1. Introduction

Beta-cypermethrin (beta-CP) [cyano-(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate] is a synthetic pyrethroid insecticide consisting of four active stereoisomers of the parent compound cypermethrin (CP). The ratio of *cis/trans* isomers in beta-CP is approximately 2/3. Synthetic pyrethroid (SP) is found to be more effective than organophosphates and carbamates, thus it has been widely used in agricultural and home formulations to control insect pests for more than 30 years and accounts for approximately one-fourth of the worldwide insecticide market. However, it is more toxic to fish and aquatic invertebrates than organophosphates and carbamates according to the reports (Van Wijngaarden et al., 2005). Beta-CP is considered as a possible human carcinogen by the Environmental Protection Agency (EPA) of USA, and also has toxicity to humans, including reproductive and developmental toxicity, neurotoxicity, and acute toxicity (Shafer et al., 2005). Its half life varies from 94.2 to 1103 days and the adsorption coefficient (Koc) is 82.1 in soil. All these factors together make it potentially harmful to health and environment.

Therefore, it is necessary to develop remediation processes to degrade and eliminate contaminants in the environment. Bioremediation, especially microbial remediation, is generally considered to be a safe and effective technique for insecticide elimination. Up to now, there are only a few reports on the biodegradation of SP insecticides such as CP by *Micrococcus* sp. strain CPN 1 (Tallur et al., 2008), allethrin by *Acidomonas* sp. (Paingankar et al., 2005) and beta-cyfluthrin (beta-CF) by *Pseudomonas stutzeri* strain S1 (Saikia et al., 2005). To our knowledge, this is the first report about beta-CP degrading microbes.

To date, most of researches have focused on the SP metabolic pathways by microorganisms (Paingankar et al., 2005; Saikia et al., 2005; Tallur et al., 2008). However, there is no report on SP uptake mode by microbes. The direct contact mode is one of the hydrophobic organic compounds uptake mechanisms. It is accepted that direct adherence to the surface of compounds is the first step in the process of removing hydrophobic organic pollutants by microbes. The bacterial cell surface hydrophobicity (CSH) is one of the most important factors that govern bacterial adhesion, uptake and degradation of hydrophobic organic compounds (Zita and Hermansson, 1997). It has been shown that bacterial cell surface hydrophobicity is better than hydrophilicity in terms of tolerance to environmental stresses (Uzel and Ozdemir, 2009). The ecological significance of the bacterial cell surface hydrophobicity was explored to supply a new theoretical basis for the bioremediation of organic pollution such as beta-CP in the environment.

The objectives of this study were to isolate and identify beta-CP degrading strains; to optimize their degrading conditions; to investigate their biodegradation ability; and then to obtain information

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whether there is a relationship between CSH and the degradation ability or not. Finally we illuminated the significance of the two strains with the high CSH on bioremediation of the beta-CP in the environment.

2. Methods

2.1. Chemicals and media

Beta-CP (98%) was procured free of charge from Shandong Huayang Technology Co., Ltd., China. 3-phenoxybenzoic acid (3-PBA) and phenol standards (98% purity) were purchased from Sigma–Aldrich, USA. Methanol and acetonitrile were of HPLC purity grade. All other chemicals used were analytical grade and purchased from Tianjin Chemical Reagent Co., Ltd., China. Beta-CP was dissolved in various solvents (including ethanol, acetonitrile, acetone, and methanol) as stock solutions, which were sterilized by membrane filtration and rationed into medium to get the desired concentrations. The enrichment medium (TYC) that contained (in gram per liter) 5 g tryptone, 5 g yeast extract, 1 g K_2HPO_4, 1 g glucose and the mineral salt medium (MSM) that contained (in gram per liter) 1 g NH_4NO_3, 1 g NaCl, 0.5 g KH_2PO_4, 1.5 g K_2HPO_4, 0.1 g $MgSO_4{\cdot}7H_2O$ were used for the isolation of bacterial strains.

2.2. Enrichment, isolation and maintenance of beta-CP degrading bacteria

Two indigenous bacterial strains were isolated from the activated sludge samples from a pesticide manufacturer in Shandong, China, which had produced carbamates and synthetic pyrethroids insecticides for many years. Enrichment and isolation of bacteria were performed as described previously (Xu and Yan, 2008). The two strains JC1 and JCN13 could make use of beta-cypermethrin as the sole carbon and energy sources to grow on the MSM. Growth was measured turbidometrically by a spectrophotometer (SCINCO S-3100) at 600 nm. The cultures were maintained on 600 mg L^{-1} beta-CP MSM at 4 °C.

2.3. Characterization and identification of isolated beta-CP degraders

Isolates were characterized and identified by morphological methods, biochemical tests and 16S rRNA gene analysis. Bergey's Manual of Determinative Bacteriology offered reference to biochemical tests (Holt et al., 1994). Genomic DNA was prepared as described previously (Sambrook and Russell, 2001). The 16S rRNA gene was amplified by PCR with IntF (AGAGTTTGATCCTGGCTCAG) and IntR (GGCTACCTTGTTACGACT) as universal primers. PCR products were cloned into a pMD18-T vector (TaKaRa).Then we transformed the plasmid to *E. coli* DH5α, screened positive clone and sent to Invitrogen Biotechnology Co., Ltd., for sequencing. The sequences which had the highest similarity with the determined 16S rRNA partial sequence by searching the GenBank database were selected and molecular evolutionary analysis were conducted using MEGA 4.0 version with the Kimura two-parameter model and the neighbor-joining algorithm (Saitou and Nei, 1987).

2.4. The optimization of the beta-CP degrading condition

Response surface methodology (RSM) based on the Box-Behnken design of experiment was used to optimize the important parameters and their interaction which significantly influenced the beta-CP biodegradation by the strain JC1 or JCN13 (Guo et al., 2009; Mohana et al., 2008). Two strains were inoculated into MSM, separately, with beta-CP (100 mg L^{-1}) as the sole carbon source and the samples were collected at the fourth day for detecting the beta-CP residues. The important factors selected for the investigation were temperature, pH and the total biomass amount. A three-variable Box-Behnken design with three replicates at the center point was applied in this experiment. The experiment design was described in Table 1. Eq. (1) shows the second-order polynomial equation.

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{j-1} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 \quad (1)$$

Y is the predicted response, X_i and X_j are variables, β_o is the constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient.

2.5. Biodegradation study

Degradation tests were carried out under the optimum conditions in MSM containing 100 mg L^{-1} beta-CP taking three replicates and the combined degradation of *cis*- and *trans*-isomers of beta-CP was detected. The bacterial growth was indicated by the optical density value by a spectrophotometer (SCINCO S-3100, Korea) at 600 nm. For the preparation of HPLC analysis, cultures were regularly harvested and extracted with acetonitrile by ultrasonic-assisted extraction (Navarro et al., 2009). It was carried out in ultrasonic crasher (SCIENTZ JY99, China) and the average recovery rate of beta-CP was 98.65%. Then, samples were dissolved in the same volume of acetonitrile and filtrated through a 0.45 μm filter. 15 μL sample was injected into a HPLC (Thermo Finnigan, USA). The analytical column was Zorbax SB-C18 column (250 × 4.6 mm, 5 μm), and the solutes were detected using PDA detector with gradient UV–VIS detection ranging from 200–600 nm. A mixture of acetonitrile and water (70:30, v/v) was used as the mobile phase at a flow rate of 1.0 mL min^{-1}. The detection wavelength of beta-CP was 278 nm.

Samples were also analyzed by GC-FID (with a HP-5 capillary column) as described previously with modifications (Liu et al., 2004), and reconfirmed on an Agilent 6890 N GC–MS system equipped with VF-5MS column (30 m × 0.25 mm × 0.25 μm). The separation parameters were as follows: the initial column temperature was 100 °C for 1 min, and ramped at 30 °C min^{-1} to 270 °C, and finally held at the temperature for 10 min. The temperature of the transfer line, ion trap and the quadrupole were 280 °C, 230 °C and 150 °C, respectively. The inlet temperature was

Table 1
Box-Behnken experimental design with three independent variables.

Run	X_1	X_2	X_3	Responses (residues of beta-CP, mg L^{-1})	
				JC1	JCN13
1	−1	−1	0	79.7	71.3
2	1	−1	0	48.8	56.2
3	−1	1	0	62.7	66.5
4	1	1	0	39.1	24.8
5	−1	0	−1	65.7	69.0
6	1	0	−1	43.7	29.9
7	−1	0	1	64.4	68.2
8	1	0	1	43.1	29.0
9	0	−1	−1	63.2	59.9
10	0	−1	1	25.9	27.7
11	0	−1	1	64.7	61.3
12	0	1	1	24.6	26.9
13	0	0	0	28.7	31.8
14	0	0	0	31.5	31.0
15	0	0	0	27.4	31.5

X_1: temperature, −1 (25 °C), 0 (30 °C), 1 (35 °C); X_2: media pH, −1 (6), 0 (7), 1 (8); X_3: biomass amount −1 (0.1 g L^{-1}), 0 (0.15 g L^{-1}), 1 (0.2 g L^{-1}).
The data was analyzed using statistical analysis system (SAS) software.
All the values were averages of three replicates from three independent experiments.