Journal of Hazardous Materials 187 (2011) 433–440

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Biodegradation of beta-cypermethrin and 3-phenoxybenzoic acid by a novel *Ochrobactrum lupini* DG-S-01

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ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 11 January 2011
Accepted 12 January 2011
Available online 18 January 2011

Keywords:
Biodegradation
Pyrethroid insecticides
Beta-cypermethrin
Ochrobactrum lupini DG-S-01
3-Phenoxybenzoic acid

ABSTRACT

A newly isolated bacterium DG-S-01 from activated sludge utilized beta-cypermethrin (beta-CP) and its major metabolite 3-phenoxybenzoic acid (3-PBA) as sole carbon and energy source for growth in mineral salt medium (MSM). Based on the morphology, physio-biochemical characteristics, and 16S rDNA sequence analysis, DG-S-01 was identified as *Ochrobactrum lupini*. DG-S-01 effectively degraded beta-CP with total inocula biomass $A_{590\,nm}$ = 0.1–0.8, at 20–40 °C, pH 5–9, initial beta-CP 50–400 mg L^{-1} and metabolized to yield 3-PBA leading to complete degradation. Andrews equation was used to describe the special degradation rate at different initial concentrations. Degradation rate parameters q_{max}, K_s and K_i were determined to be 1.14 d^{-1}, 52.06 mg L^{-1} and 142.80 mg L^{-1}, respectively. Maximum degradation was observed at 30 °C and pH 7.0. Degradation of beta-CP was accelerated when MSM was supplemented with glucose, beef extract and yeast extract. Studies on biodegradation in liquid medium showed that over 90% of the initial dose of beta-CP (50 mg L^{-1}) was degraded under the optimal conditions within 5 d. Moreover, the strain also degraded beta-cyfluthrin, fenpropathrin, cyhalothrin and deltamethrin. These results reveal that DG-S-01 may possess potential to be used in bioremediation of pyrethroid-contaminated environment.

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1. Introduction

Beta-cypermethrin (beta-CP) [cyano-(3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate], is one of the important pyrethroid insecticides (PIs) used for insect control both in agricultural and home formulations. PIs are frequently and widely used throughout the world, especially in recent years with the restrictions or eliminations of highly toxic organophosphates or carbamates and account for approximately one quarter of the global insecticide market [1,2]. Although PIs were generally considered safe for humans yet some researches showed that they might have cumulative toxicity [3], reproductive toxicity [4] and poisoning of nervous system in non-target creatures [5,6]. Long-term exposure to PIs may lead to some chronic diseases [7]. Moreover, beta-CP is regarded as a possible human carcinogen by the Environmental Protection Agency (EPA) of USA [8]. The reported half-life of beta-CP in soil ranges from 94.2 to 1103 d, with 3-phenoxybenzoic acid (3-PBA) as the major degradation product [8]. 3-PBA has greater soil mobility than the parent compound and causes widespread contamination in soils. It was suggested that 3-PBA was not only persistent towards degradation by microorganisms but also limited the biodegradation of beta-CP due to its antimicrobial activities [9]. Moreover, it possesses antiestrogenic activity being considered as endocrine disruptors [10–12]. With the persistent use of beta-CP, ecological safety and health risk were increasingly serious.

Therefore, it is necessary to develop remediation strategies to degrade and eliminate beta-CP and 3-PBA from the environment. Biodegradation involves the use of living microorganisms to detoxify and degrade hazardous materials, and is generally considered to be an effective and safe way to remove contaminants from environment. Biodegradation has been applied widely in degrading environmental pollutants such as pesticides, petroleum, plastic, dye, surface active agent, etc. Some bacterial strains such as *Bacillus* sp. [13], *Pseudomonas* sp. [14], *Alcaligenes* sp. [15], *Aspergillus* sp. [16], *Acidomonas* sp. [17], *Micrococcus* sp. [18], *Sphingobium* sp. [19], and *Klebsiella* sp. [20] have been reported to degrade PIs. However, there are rare reports about bacterial strains that can degrade both beta-CP and 3-PBA.

In most cases reported to date, the degrading bacteria tended to transform PIs by hydrolysis to produce 3-PBA, which in turn accumulated in the medium or soils and enhanced degradation could not occur. Studies on further metabolite were not extensive. In the present research, a novel bacterial strain that could degrade both

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doi:10.1016/j.jhazmat.2011.01.049

beta-CP and 3-PBA was isolated and optimization of the conditions for biodegradation was investigated.

2. Materials and methods

2.1. Chemicals and media

Technical grade beta-CP (94%), beta-cyfluthrin (98%), fenpropathrin (92.5%), cyhalothrin (95%) and deltamethrin (95%) used in this study were obtained from Syngenta (China) Investment Co. Ltd. 3-PBA standard (98%) and HPLC grade acetonitrile were purchased from Sigma–Aldrich, USA. Beta-CP and 3-PBA were dissolved in acetone as stock solutions (100,000 mg L^{-1}), which were sterilized by membrane filtration and rationed into medium to get the desired concentrations. All other chemicals and solvents used were of analytical grade and purchased from Merck, Germany. The mineral salt medium (MSM) containing 2.0 g of $(NH_4)_2SO_4$, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $CaCl{\cdot}2H_2O$, 0.001 g of $FeSO_4{\cdot}7H_2O$, 1.5 g of $Na_2HPO_4{\cdot}12H_2O$, and 1.5 g of KH_2PO_4 per liter of water and nutrient broth medium (NBM) containing 0.5% beef extract, 1% tryptone and 0.5% NaCl per liter of water was used. The final pH was adjusted to 7.0.

2.2. Enrichment, isolation and screening of bacterial strains

The bacterium was isolated using an enrichment culture technique. Activated sludge samples were collected as inoculum from a pyrethroid-manufacturer in Zhongshan, China. Enrichment and isolation of degrading strains were performed as described in detail previously [21]. DG-S-01 that utilized beta-CP and 3-PBA as sole carbon and energy source for growth in MSM was selected for further studies.

2.3. Taxonomic identification of DG-S-01

DG-S-01 was identified by the morphology, physio-biochemical characteristics, and 16S rDNA gene analysis. Cell morphology was observed with electron microscope (Olympus, Japan). Physio-biochemical tests were examined with reference to Bergey's Manual of Determinative Bacteriology [22]. Genomic DNA was prepared according to standard methods [23]. 16S rDNA gene was amplified using universal primers, B1 (5′-AGAGTTTGATCCTGGCTCAG-3′, *Escherichia coli* bases 8–27) and B4 (5′-ACGGHTACCTTGTTTACGACTT-3′, *E. coli* bases 1507–1492) [24]. PCR product containing the amplified 16S rDNA gene fragment was purified with QIAquick Gel Extraction Kit spin column (Guangzhou Heda Technology Co. Ltd., China), ligated to the linear vector pMD20-T (TaKaRa Biotechnology Co. Ltd., China) and transformed into *E. coli* DH5α cells. Positive clones were screened and sent to Shanghai Yingjun Technology Co. Ltd., China, for sequencing. The resulting sequence was compared with the genes available in the GenBank nucleotide library by a BLAST search through the National Center for Biotechnology Information (NCBI). Multiple alignments of 16S rDNA were carried out using CLUSTALX 1.8.1 and phylogeny was analyzed using MEGA 4.0 [25]. An unrooted tree was built using the neighbor-joining method.

2.4. Inoculum preparation

To prepare the inoculum, pure culture of isolate, obtained from individual colonies, was inoculated to NBA plates and incubated at 30 °C. After 24 h cultivation, the isolate was transferred into NBM, harvested by centrifugation at 4000 rpm for 2 min, washed with 0.9% sterile N-saline and resuspended in N-saline to set an $A_{590\,nm}$ of 0.6. Colony forming units (CFU mL^{-1}) of this suspension were quantified by the dilution plate count technique. One percent of this suspension (approximately 1.0×10^8 cells mL^{-1}) was used as inoculum for beta-CP and 3-PBA biodegradation studies.

2.5. Effect of alternate carbon source/nutrients on degradation of beta-CP by DG-S-01

To study the effect of extra carbon source or nutrients on the bacterial degradation rate of beta-CP, the MSM (50 mL) with addition of 1% (w/v) carbon source or nutrients (glucose, beef extract and yeast extract) was used. Beta-CP was introduced in form of acetone solution to give the final concentration of 50 mg L^{-1}. The 250 mL Erlenmeyer flasks (in triplicate) were incubated at 30 °C and 180 rpm on a rotary shaker for 5 d and pesticide concentration was determined at 24 h interval. Triplicate samples for MSM + beta-CP + carbon source or nutrients, non-inoculated with the bacterial suspension were kept as controls.

2.6. Optimal conditions for degrading beta-CP by DG-S-01

To determine the optimal conditions for degrading beta-CP by DG-S-01, single-factor test was designed in this study under different conditions of inoculum amount ($A_{590\,nm}$ was set 0.1–0.8), initial concentration of beta-CP (50–400 mg L^{-1}), temperature (20–40 °C) and pH (5.0–9.0). DG-S-01 was incubated in NBM containing 50 mg L^{-1} beta-CP at 30 °C and 180 rpm on a rotary shaker. Each treatment was set in triplicate with non-inoculated samples as control. Residual pesticide concentration was determined at 24 h interval.

2.7. Degradation kinetics of various substrates by DG-S-01

The abilities of DG-S-01 to degrade different PIs were determined under the optimal degradation conditions. The sterilized liquid media were supplemented with beta-CP, beta-cyfluthrin, fenpropathrin, cyhalothrin and deltamethrin at 50 mg L^{-1}, respectively, and incubated at 180 rpm on a rotary shaker for 5 d. The experiment was conducted in triplicate with non-inoculated samples as control. The sampling was carried out at interval of 24 h and the pesticide residues were measured by HPLC.

2.8. Degradation of 3-phenoxybenzoic acid (3-PBA) by DG-S-01

Different concentrations of 3-PBA (25 mg L^{-1}, 50 mg L^{-1}, and 100 mg L^{-1}) in MSM were inoculated with DG-S-01 and incubated at 30 °C and 180 rpm on a rotary shaker. Each treatment was performed in triplicate with non-inoculated samples as controls. 3-PBA residues were determined at 1, 3, 5, 7, and 9 d.

2.9. HPLC conditions and analysis

Samples were extracted and performed on an Agilent 1100 HPLC equipped with a ternary gradient pump, programmable variable-wavelength UV detector, column oven and electric sample valve and C_{18} reversed-phase column (Hypersil ODS2 5 μm × 4.6 mm × 250 mm). The analyses of PIs were performed using a mobile phase of 85:15 acetonitrile and water. Sample injection volume was 10 μL, mobile phase was programmed at a flow rate of 1 mL min^{-1}. PIs were detected at 235 nm wavelengths [26]. The retention time for beta-CP, beta-cyfluthrin, fenpropathrin, cyhalothrin and deltamethrin under these chromatographic conditions was 6.4 min, 5.9 min, 6.2 min, 6.6 min and 7.0 min, respectively.

The metabolite 3-PBA was also analyzed by HPLC and a mixture of acetonitrile and water (60:40, v/v) was used as the mobile phase at a flow rate of 1 mL min^{-1}. pH value of water was adjusted to 2.4 using phosphoric acid before mixture [27]. 20 μL samples

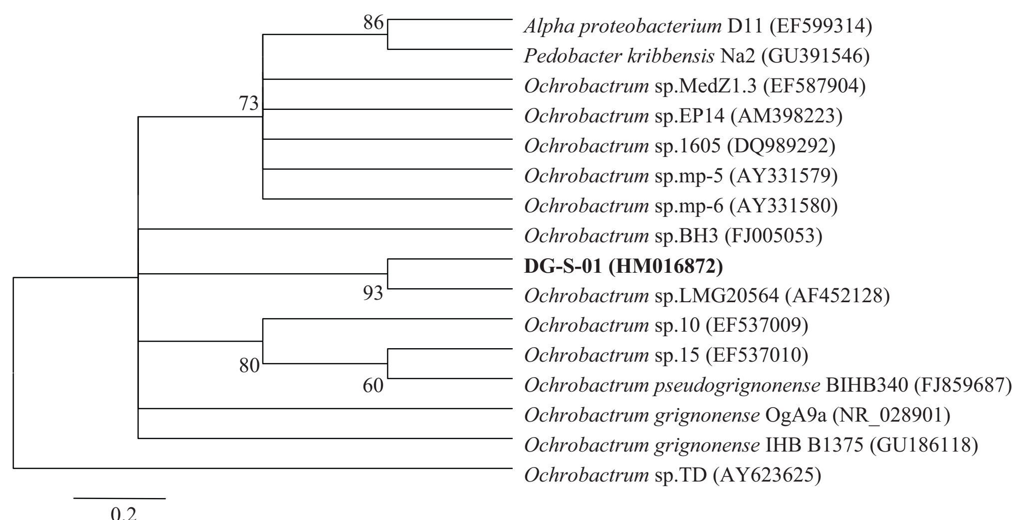

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on 16S rDNA sequences of DG-S-01 and related strains. Bootstrap values are given at branching points.

were injected into HPLC and detection wavelength was 220 nm. Retention time was found to be 5.3 min for 3-PBA.

3. Results and discussion

3.1. Isolation and screening of beta-CP degrading bacterium

According to the results of soil enrichment culture 10 morphologically different strains (grown on NBA containing 500 mg L^{-1} beta-CP) were attained by streaking plate method and named as DG-S-01–10, respectively. The results showed that DG-S-01 possessed the highest degradation capacity among 10 strains. DG-S-01 utilized approximately 87% beta-CP within 7 d of incubation. Moreover, DG-S-01 was also found to efficiently utilize beta-cyfluthrin, fenpropathrin, cyhalothrin, and deltamethrin as growth substrate besides beta-CP. Therefore, DG-S-01 was selected for further studies.

In the present study, beta-CP was used in the screening process to isolate some potential isolates with a high survivability in the environment and maximal degrading activity towards beta-CP. It was generally considered that the conditions for environmental microorganisms enrichment and screening are crucial in the selection of isolates with the desired degrading enzyme systems and having specific regulation of the degradation pathways as well [28].

3.2. Identification and characterization of DG-S-01

3.2.1. Basic morphology, physio-biochemical characteristics of DG-S-01

Colonies of DG-S-01 appeared circular and light yellow with regular or margin during growth on NBA plates for 48 h. Cells of DG-S-01 were motile and small rod shaped with 3.1324 μm in length and 1.9505 μm in width. It was positive in tests such as triple sugar iron (TSI), methyl red (MR), starch hydrolysis, milk coagulation, nitrate reduction, hydrogen sulfide production and utilized simmons citrate, lactose, glucose, maltose, amylum, D-galactose, D-fructose, D-xylose, saccharose and rhamnose. It was negative in Gram staining, fibrinolysis, Voges–Proskauer (V–P), indole reaction, gelatin liquefaction and utilization of D-fucose and mannose.

3.2.2. Sequence analysis of 16S rDNA of DG-S-01

PCR amplification of 16S rDNA gene from DG-S-01, a single fragment of 1445 bp, GenBank accession no. HM016872, was obtained and completely sequenced. According to BLAST analysis, the resulting sequence had high similarity to the 16S rDNA gene sequence of bacteria belonging to *Ochrobactrum* group and closely clustered with strain LMG20564 (GenBank accession no. AF452128) and strain EP14 (GenBank accession no. AM398223), with sequence identities of 99% and 98%, respectively. A phylogenetic tree was constructed based on the 16S rDNA gene sequence of DG-S-01 and related strains using MEGA 4.0 (Fig. 1). In combination with the morphology, physio-biochemical characteristics and 16S rDNA gene analysis, DG-S-01 was tentatively identified as *Ochrobactrum lupini*.

Bacteria from genera *Pseudomonas* and *Bacillus*, known as metabolically active microorganisms, capable of degrading many pesticides were isolated from various soils polluted with cypermethrin and other PIs, such as cyfluthrin, biphenthrin, fenvalerate, fenpropathrin [13,14], while *O. lupini* appears to be a new bacterium genus that may participate in efficient degradation of beta-CP and other PIs. To our knowledge, there is not any information concerning the ability to degrade beta-CP and other PIs by bacteria belonging to *Ochrobactrum* sp. However, studies showed that *Ochrobactrum* sp. could effectively degrade organophosphorus pesticides [29].

3.3. Utilization of beta-CP as sole source of carbon for growth by DG-S-01

The *O. lupini* DG-S-01 utilized beta-CP as sole carbon and energy source in MSM and growth was the most optimum after 2 d of incubation, as shown in Fig. 2. Under the optimum conditions, DG-S-01 degraded beta-CP rapidly reaching up to 80% of the initial dose within 5 d (Fig. 2). Finally, 50 mg L^{-1} beta-CP was completely degraded after 10 d of incubation. In biodegradation assay, degradation of beta-CP was accompanied by transient accumulation of 3-PBA, but it was subsequently degraded rapidly and disappeared finally. All degradation was associated with bacterial growth as described previously [30]. It was reported that addition of pesticide as the sole carbon sources led to a lag phase followed by enhanced biodegradation [21]. In our studies, however, the bacterium adapted quickly to the environment and degraded beta-CP rapidly at the beginning of incubation without any apparent lag phase. The results indicated that DG-S-01 might possess potential to be used in bioremediation of beta-CP contaminated environment.

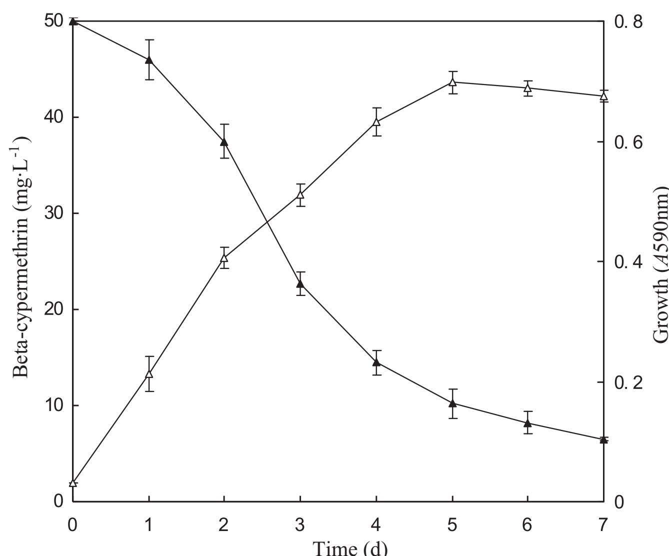

Fig. 2. Utilization of beta-cypermethrin (▲) during growth (△) of DG-S-01. *Note*: values are means ± SD of three replicates. The following figures are the same.

3.4. Effect of alternate carbon source/nutrients on degradation of beta-CP

In this experiment, glucose, beef extract and yeast extract were used as additional carbon source. The curves of beta-CP degradation were different as compared to degradation in MSM without additional carbon source. The beta-CP degradation significantly enhanced at the presence of glucose, beef extract and yeast extract, reaching to 85.4%, 90.4% and 87.0% within 5 d of incubation, respectively, while in the same period disappearance rate of beta-CP in MSM without carbon source was lower and only reached to 79.6% (Fig. 3). A non-inoculated control showed no change in degradation after 5 d. This might be because of the cometabolism with other carbon sources that could obviously improve the disappearance rate of beta-CP in liquid medium. However, this result contrasts with previous findings that reported addition of other carbon sources led to a lag phase followed by accelerated biodegradation [28,30]. Our findings revealed that DG-S-01 preferred to utilize beta-CP even in the presence of carbon rich environment and its degradation ability was positively influenced by the presence of the supplementary carbon sources and nutrients. Similar enhanced degradation at the presence of carbon sources and nutrients was observed by Anwar et al. [21].

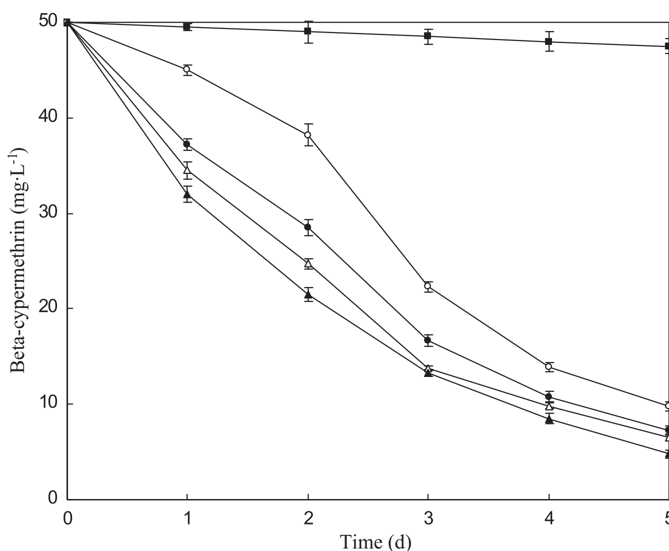

Fig. 3. Degradation of beta-cypermethrin by DG-S-01 in mineral salt medium (MSM) containing 50 mg L^{-1} beta-cypermethrin as sole source of carbon and energy (○) and in the presence of other nutrients, i.e. glucose (●), yeast extract (△), beef extract (▲), and control without inoculum (■).

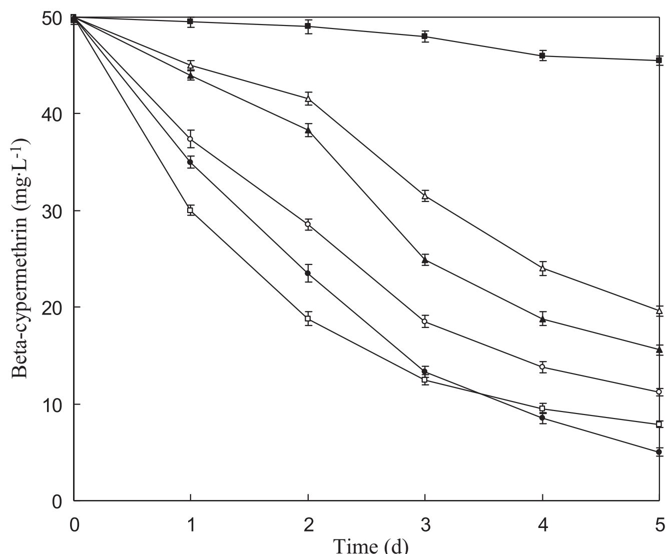

Fig. 4. Degradation of beta-cypermethrin by DG-S-01 in nutrient broth medium (NBM) containing 50 mg L^{-1} beta-cypermethrin at various inoculum amounts, i.e. $A_{590\,nm}$ = 0.1 (△), $A_{590\,nm}$ = 0.2 (▲), $A_{590\,nm}$ = 0.4 (○), $A_{590\,nm}$ = 0.6 (●), $A_{590\,nm}$ = 0.8 (□), and control without inoculum (■).

3.5. Effect of inoculum amount on degradation of beta-CP

It was reported that inoculum amount was an important factor determining the efficient biodegradation of pesticides [31,32]. Our results also supported the view and showed that the effect enhanced with the increasing of the inoculum amount and duration of time in certain extent responding to degradation of pesticides (Fig. 4). The results indicated that inoculated with highest initial cell density $A_{590\,nm}$ = 0.8, degradation of beta-CP by DG-S-01 started rapidly within 1 d of incubation and more than 75% of beta-CP was degraded within 3 d. During the first incubation period (0–3 d), the disappearance rates of beta-CP at $A_{590\,nm}$ = 0.1, 0.2, 0.4, and 0.6 were lower than that of $A_{590\,nm}$ = 0.8, which might be because that greater number of bacteria was needed to initiate rapid degradation of pesticide during incubation. It has been observed that when lower inoculum amount was used, only a small part of introduced bacteria survived the initial competition and participated in pesticide degradation [33]. A higher initial inoculum could compensate for the initial population declined and survivors could multiply and degrade xenobiotics [34]. Another possible reason for enhanced degradation with the increase of inoculum amount could be due to the fact that the survival microorganisms might use dead microbial structures as a nutrient supply. However, the curve with highest initial cell density $A_{590\,nm}$ = 0.8 tended to be steady after 3 d in our studies. It may be because increasing of the inoculum lead to relative shortage of carbon and the strains competed mutually the limited resource. In contrast, it was shown that further degradation on beta-CP was still preformed by DG-S-01 when incubated with lower inoculum amounts after 3 d. Finally, 60.8%, 69.0%, 77.6%, 90.0%, and 84.2% of the initial beta-CP dose was degraded at $A_{590\,nm}$ = 0.1, 0.2, 0.4, 0.6, and 0.8 after 5 d, respectively.

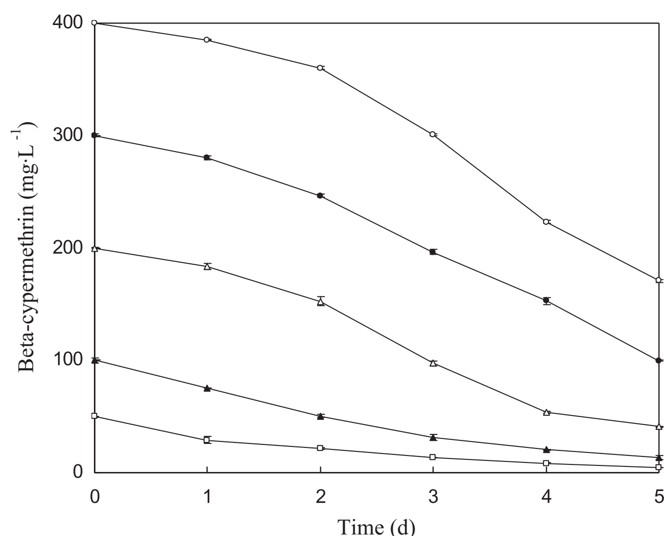

Fig. 5. Degradation of beta-cypermethrin by DG-S-01 in nutrient broth medium (NBM) at concentrations 50 mg L^{-1} (□), 100 mg L^{-1} (▲), 200 mg L^{-1} (△), 300 mg L^{-1} (●), and 400 mg L^{-1} (○). Values are means of three replicates with standard deviation.

3.6. Effect of initial concentration of beta-CP on its degradation

Fig. 5 shows the kinetic curves of beta-CP degradation by DG-S-01 at different initial concentrations by strain DG-S-01. At low beta-CP concentration (50–200 mg L^{-1}), the degradation rate reached 80% within 5 d, especially at less than 100 mg L^{-1}. However, at high concentration (300–400 mg L^{-1}), only 60% was achieved within 5 d. It might be because of the fact that microbial degradation starts slowly and requires an acclimation period before rapid degradation occurs at high concentration. The results are consistent with previous findings of Cycon et al. [32] who reported that initial dose of diazinon was efficiently degraded by strain *Serratia* sp. and *Pseudomonas* sp. in soil. Furthermore, longer lag phase was observed with increasing concentration of beta-CP in our studies as described earlier [21]. These results are also similar with previous findings of Karpouzas and Walker [35] who revealed that longer lag phase at higher concentration might be because of that the greater number of bacteria was needed to initiate rapid degradation of pesticide.

To determine the effect of initial beta-CP concentration on the degradation increase, Andrews equation was used to describe the specific degradation rate (q) at different initial concentrations [36,37].

$$q = \frac{q_{\max} S}{S + K_s + (S^2/K_i)}$$

where $q_{\max}$ is the maximum specific degradation rate, K_i is the inhibition constant, K_s is the half-rate constant, and S is the beta-CP concentration.

The relationship between specific degradation rate (q) and initial beta-CP concentration is shown in Fig. 6, and the parameters q_{max}, K_s and K_i were determined to be 1.14 d^{-1}, 52.06 mg L^{-1} and 142.80 mg L^{-1}, respectively. The value of R^2 was 0.9175 demonstrating that the experimental data was well correlated with Andrews equation. As shown in Fig. 6, when the initial concentrations of beta-CP were lower than 80 mg L^{-1}, the q gradually increased. At higher concentrations above 100 mg L^{-1}, the inhibition effect of beta-CP became prominent. It is a fact that beta-CP displays the inhibitory nature at high concentrations.

3.7. Effect of temperature on degradation of beta-CP

Beta-CP was degraded by DG-S-01 during incubation with all the five temperatures (20–40 °C) tested. In cultures incubated at 30 °C and 35 °C, beta-CP degradation started rapidly within 1 d, apparently there was no lag phase and more than 85% of beta-CP was degraded after 5 d of incubation (Fig. 7). However, in cultures incubated at higher or lower temperature, i.e. 20 °C, 25 °C and 40 °C the disappearance rates of beta-CP only reached 53.6%, 75.8%, and 67.6% within 5 d, respectively. These results were consistent with previous findings of Zhao et al. [38] who reported temperature significantly influenced the biodegradation of wainsonine by *Acinetobacter calcoaceticus* strain YLZZ-1. Our results also indicated that maximum degradation of beta-CP was achieved at 30–35 °C revealing that the strain preferred relatively high temperature

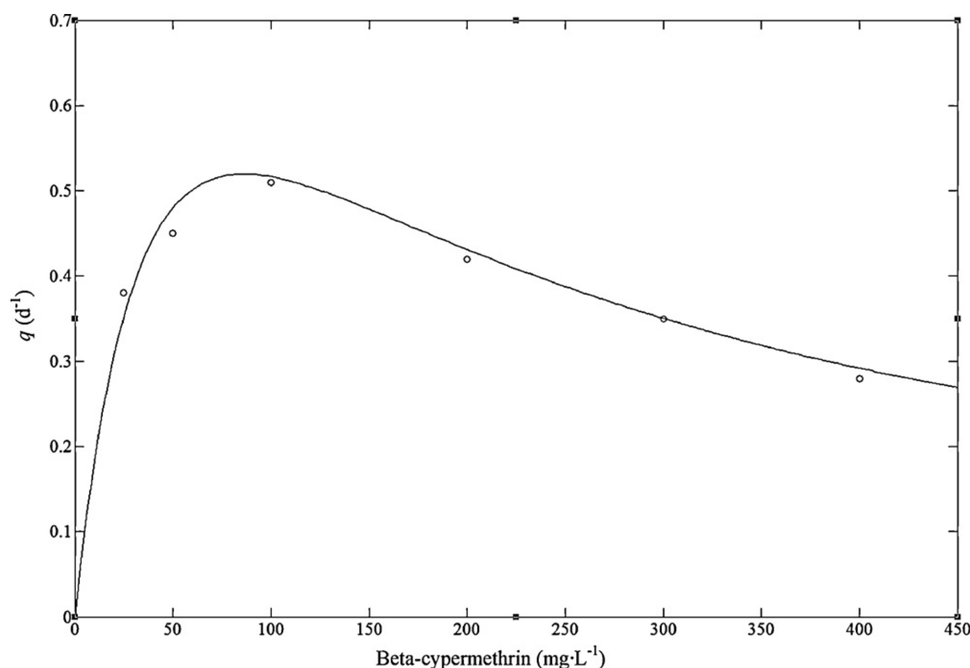

Fig. 6. Relationship between specific degradation rate and initial beta-cypermethrin concentration by DG-S-01.

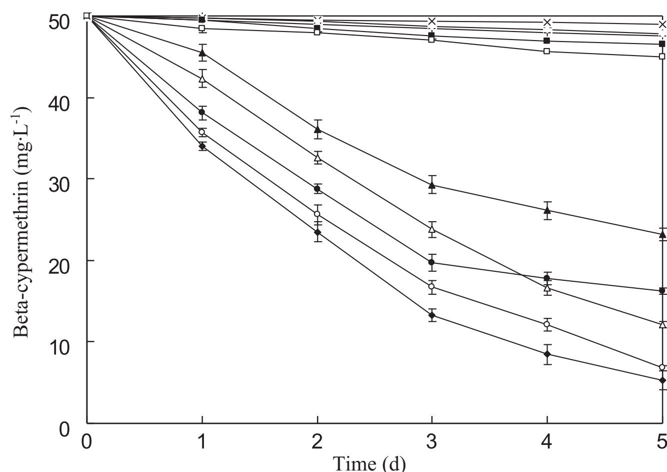

Fig. 7. Degradation of beta-cypermethrin by DG-S-01 in nutrient broth medium (NBM) containing 50 mg L^{-1} beta-cypermethrin at various temperatures, i.e. 20 °C (▲), 25 °C (△), 30 °C (♦), 35 °C (○), 40 °C (●), and control without inoculum at 20 °C (×), 25 °C (◊), 30 °C (+), 35 °C (■), and 40 °C (□).

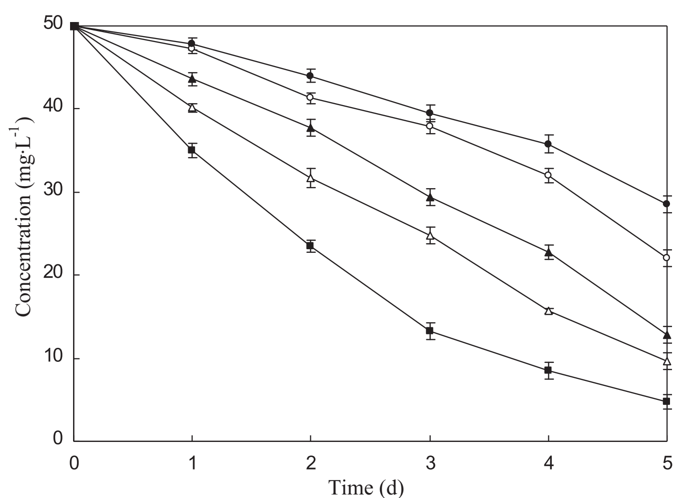

Fig. 9. Degradation capacity of different pyrethroids by DG-S-01 in nutrient broth medium (NBM) with the final concentration of 50 mg L^{-1}, i.e. beta-cypermethrin (■), beta-cyfluthrin (△), fenpropathrin (▲), cyhalothrin (○), and deltamethrin (●).

environment. Similar results were reported by Zhang et al. [8]. It is possible that some key enzyme(s) responsible for beta-CP degradation have their optimum enzymatic activity over such range of temperature. In non-inoculated controls at different temperatures, abiotic degradation was negligible throughout the studies.

3.8. Effect of pH on degradation of beta-CP

The pH value is another important factor which significantly influences the degradation ability of microorganisms capable of degrading xenobiotics [39,40]. This study determined the effect on degradation activity of beta-CP by DG-S-01 at different pH conditions, i.e. 5.0, 6.0, 7.0, 8.0, and 9.0. It showed 56.8%, 63.4%, 89.8%, 83.0%, and 79.4% of degradation rate within 5 d, respectively (Fig. 8). Initial pH value of 7.0 was found to be optimum for degradation. Results indicated that DG-S-01 was capable of degrading beta-CP rapidly over a wide range of pH particular at pH as low as 5.0. This is an important feature of an organism to be employed for bioremediation of variable environments [21]. However, these results contrasted with previous findings of Zhang et al. [8] who reported

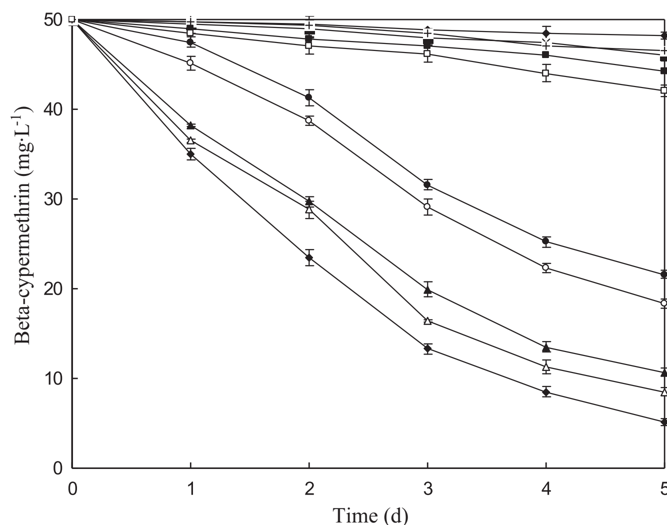

Fig. 8. Degradation of beta-cypermethrin by DG-S-01 in nutrient broth medium (NBM) containing 50 mg L^{-1} beta-cypermethrin at various pH, i.e. pH = 5.0 (●), pH = 6.0 (○), pH = 7.0 (♦), pH = 8.0 (△), pH = 9.0 (▲), and control without inoculum at pH = 5.0 (◊), pH = 6.0 (+), pH = 7.0 (×), pH = 8.0 (■), and pH = 9.0 (□).

rapid degradation of beta-cypermethrin by two *Serratia* spp. at high pH while it was very low at acidic pH. Our results also revealed that the beta-CP degradation was liable to happen at neutral and alkaline trending condition. This may be because neutral and alkaline trending condition contributes to synthesis and expression of degradation enzyme of strain DG-S-01. Similar enhanced degradation at neutral and alkaline trending condition was observed by previous reports [21]. In non-inoculated controls at different pH conditions, abiotic degradation was negligible throughout the studies.

3.9. Degradation kinetics of various substrates by DG-S-01

Fig. 9 shows the degradation kinetics of different PIs by DG-S-01 in NBM. The strain was capable of degrading beta-CP, beta-cyfluthrin, fenpropathrin, cyhalothrin and deltamethrin even in the presence of other carbon sources. The degradation rates reached up to 90.4%, 80.8%, 74.4%, 56.2%, and 43.0% within 5 d, respectively. Zhang et al. [8] reported that *Serratia* sp. strain JC1 could degrade 92% beta-CP within 10 d. Grant et al. [14] reported that *Pseudomonas* sp. could degrade 90% cypermethrin within 20 d. In the present studies, the disappearance rate of beta-CP by DG-S-01 got over 90% in a much shorter time. Furthermore, DG-S-01 also degraded other pyrethroids while *Serratia* sp. strain JC1 and *Pseudomonas* sp. could not. Obviously, the degrading efficiency of DG-S-01 was higher than that of *Serratia* sp. strain JC1 or *Pseudomonas* sp. The results also revealed that beta-CP was the most preferred substrate; beta-cyfluthrin and fenpropathrin were degraded relatively slower but much faster than cyhalothrin and deltamethrin. Deltamethrin was found to be the most persistent. The results indicated that chemical and structural properties of pesticides significantly affected efficient degradation by DG-S-01. Similarly, Zhang et al. [41] confirmed that a recombinant *Strenotrophomonas* sp. was affected mostly by many physical, chemical and structural properties of both the contaminants and the soil matrix in actual field-scale bioremediation. As described earlier, bioremediation process was strongly influenced by environmental factors such as substrate, nutrients status, dwelling microorganisms, pH, temperature and biotic factors like inoculum amount [40].

To determine the effect on degradation of different PIs, the first-order model was used to express the kinetics [32,42].

$$C_t = C_0 \times e^{-kt}$$

Table 1
Kinetic parameters of degradation of different pyrethroids by DG-S-01.

Treatment	Equation	k (d^{-1})	$T_{1/2}$ (d)	R^2
Beta-cypermethrin	$y = 53.3389e^{-0.3672x}$	0.3672	1.9	0.9402
Beta-cyfluthrin	$y = 52.9232e^{-0.3073x}$	0.3073	2.3	0.9482
Fenpropathrin	$y = 52.5700e^{-0.2607x}$	0.2607	2.7	0.9448
Cyhalothrin	$y = 50.3675e^{-0.1066x}$	0.1066	6.5	0.9894
Deltamethrin	$y = 50.1503e^{-0.0840x}$	0.0840	8.2	0.9970

Note: y = residual concentration of pyrethroids (mg L^{-1}); x = degradation period (d). R^2 = correlation coefficient.

where C_0 is the initial concentration of substrate at time zero, C_t is the concentration of substrate at time t, k and t are the degradation rate constant (d^{-1}) and degradation period in days, respectively. The degradation half-lives ($T_{1/2}$) of different substrates were determined using the algorithm.

$$T_{1/2} = \frac{\ln 2}{k}$$

As shown in Table 1, the degradation rate constant (k) of each substrate calculated ranged from 0.0840 to 0.3672 d^{-1} with degradation half-lives ($T_{1/2}$) of 1.9 to 8.2 d, respectively. The values of R^2 were determined to be 0.9402–0.9970. It meant that the degradation data collected, fit well with the model.

3.10. Biodegradation of 3-phenoxybenzoic acid (3-PBA)

3-Phenoxybenzoic acid (3-PBA) is a major metabolite of pyrethroids except cyfluthrin [43]. In this study, DG-S-01 was inoculated in MSM containing 25, 50, and 100 mg L^{-1} 3-PBA. It was found that over 90% of 3-PBA (25–50 mg L^{-1}) was degraded within 9 d (Fig. 10). However, the disappearance rate of 3-PBA only reached 84.7% with initial concentration of 100 mg L^{-1} after 9 d of incubation. Differential sampling was performed while degrading 3-PBA with DG-S-01. The reaction was initiated with a 3-PBA concentration of 25 mg L^{-1} and performed for 14 d. There was an apparent peak at a retention time of around 5.3 min corresponding with 3-PBA standard. Along with the degradation of 3-PBA, the peak area at 5.3 min decreased. Eventually, 3-PBA was completely degraded by DG-S-01 after two weeks. It has been reported that the metabolite 3-PBA from the degradation of PIs had the effects of antimicrobial activities thus enhanced degradation of PIs could not occur [9,10,27,44]. However, in our studies degradation of beta-CP and 3-PBA was both achieved by the same strain. To our knowledge, this is the first report that strain DG-S-01 tolerated and degraded high concentration of 3-PBA (100 mg L^{-1}). In previous studies, Topp and Akhtar [9] reported a *pseudomonas* strain that utilized 3-phenoxybenzoate and converted it to phenol. Halden et al. [27] introduced three *Pseudomonas* sp. strains into soil microcosms containing 3-PBA and found that they used 3-PBA as a growth substrate. However, all these strains could not degraded beta-CP. Owing to its antimicrobial activities, biodegradation of 3-PBA was rarely reported. In the present studies, DG-S-01 was found to degrade not only beta-CP but also its major metabolite 3-PBA. Moreover, during biodegradation studies of beta-CP (Sections 3.3–3.8) under various conditions, no accumulation of 3-PBA was observed indicating the efficiency of this strain to degrade both beta-CP and 3-PBA.

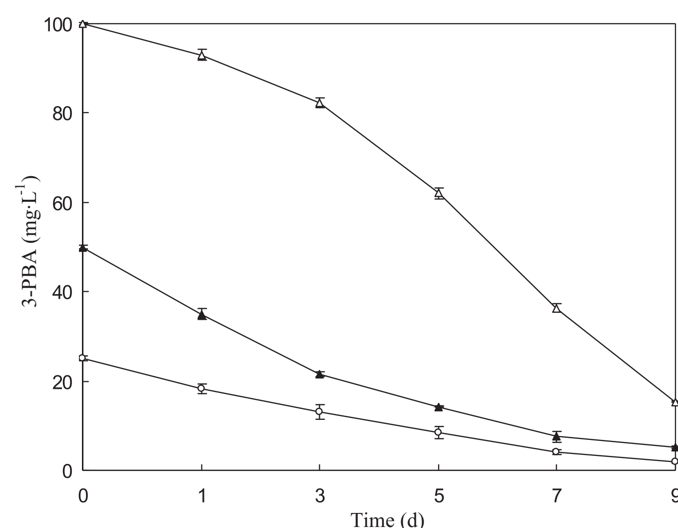

Fig. 10. Degradation of 3-phenoxybenzoic acid (3-PBA) by DG-S-01 in mineral salt medium (MSM) at concentrations 25 mg L^{-1} (○), 50 mg L^{-1} (▲), and 100 mg L^{-1} (△).

4. Conclusions

In this study, an enrichment procedure allowed us to isolate a novel bacterial strain named DG-S-01 belonging to *O. lupini* that might participate in efficient degradation of beta-CP and other pyrethroids. DG-S-01 was capable of rapidly degrading beta-CP without a lag phase over a wide range of pH and temperature. This is a very important feature of an organism to be employed for bioremediation of variable environments. It is noteworthy that DG-S-01 tolerated and degraded beta-CP up to the concentration, as high as 400 mg L^{-1}. Furthermore, this particular strain was also found highly effective in degrading 3-PBA, which is a major metabolite from most of the pyrethroids. Degradation of this compound by the same strain that degraded pyrethroids was very important because 3-PBA is not only persistent to biodegradation but also limits the biodegradation of the pyrethroids due to its antimicrobial activities. Results of the study depicted that DG-S-01 was proficient for biodegradation and implied for developing a bioremediation strategy of pyrethroid-contaminated environment. However further studies, such as its interaction with environment, biochemical and genetic aspects, are still needed before the application of this strain in the field-scale bioremediation.

Acknowledgments

This work was supported by the National Natural Science Foundation, PR China (no. 30871660) and Project of Scientific Technological Planning of Guangdong Province, PR China (no. 2009B020310005).

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