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ORIGINAL ARTICLE

Biodegradation of methidathion by *Serratia* sp. in pure cultures using an orthogonal experiment design, and its application in detoxification of the insecticide on crops

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Abstract An enrichment culture method was applied to the isolation of a bacterial strain responsible for biodegradation of methidathion residues, from a methidathion-treated orchard. The strain (SPL-2) was identified as *Serratia* sp. according to its physiological characteristics and 16S rRNA gene phylogenetic analysis. *Serratia* sp. was able to grow in a poor medium consisting of mineral salts and using methidathion as the sole carbon source at a concentrations of 50–150 mg/L. The effects of multifactors on degradation of methidathion in pure cultures by *Serratia* sp. were investigated using an orthogonal experimental design L_9 (3^4). On the basis of range analysis and ANOVA results, the most significant factors were temperature and inoculum size. The optimal conditions for methidathion biodegradation in pure cultures were a temperature in 30 °C, an inoculum size of 10 %, pH=7 and an aeration rate of 200 rpm. Two different concentrations of strain SPL-2 fermenting liquids (OD_{600}= 0.2 and OD_{600}=0.4) were prepared and applied to remove methidathion residues from agricultural products, and this process can be described by a first order rate model. In contrast to controls, the DT_{50} of methidathion was shortened by 35.7 %, 8.2 % and by 62.3 %, 57.5 % on OD_{600}=0.2 and OD_{600}=0.4 treated haricot beans and peaches, respectively. These results suggest that the isolated bacterial strain may have potential for use in bioremediation of methidathion-contaminated crops.

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Introduction

In recent years, the use of organophosphate insecticides in agricultural practice has increased dramatically. Organophosphates have gradually replaced organochlorine compounds in the protection of agricultural products due to their relatively low persistence and toxicity in the environment. However, most organophosphate insecticides do not reach their target organisms completely. Instead, they persist on the surface of agricultural products or are released directly to the environment, causing contamination of soil and water resources. This can lead to a variety of negative effects in nontarget species, including humans (Abdollahi et al. 2004; Das and Mukherjee 2000) as organophosphates can act as inhibitors of acetylcholinesterase (AChE) activity, which is responsible for the degradation of the neurotransmitter cetylcholine. To produce high-quality products in large yields, pesticides are applied extensively to the cultivated land—the major source of agricultural products. Therefore, high levels of pesticide residues on the surface of agricultural products could cause serious environmental pollution and threaten consumer health due to consumption of contaminated products.

Among the various organophosphorous pesticides, methidathion [O,O-dimethylS-(2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl) phosphorodithioate] is a non-systemic insecticide and acaricide most commonly used to control sucking and chewing insects in agriculture, and has been listed as class I toxic chemical by the United Stated Environmental Protection Agency (US EPA) because of its high toxicity. According to previous reports, methidathion is categorized as a carcinogen (Quest et al. 1990) and can cause

liver damage (Altuntas et al. 2002; Sutcu et al. 2006), vascular wall damage (Yavuz et al. 2005) as well as nephrotoxicity (Sulak et al. 2005) in rats. A considerable number of literature reports also indicate that methidathion has been found in treated agricultural products and derived food commodities, such as oranges and tangerines (Blasco et al. 2006), wine, fruit juices (Zambonin et al. 2004), nectarine and spinach (Cervera et al. 2010), olive oil (Fuentes et al. 2008), and so on. Therefore, establishment of an efficient, safe and cost-effective measure is sorely needed in order to remove and detoxify methidathion residues in contaminated food and the environment.

According to published research, methidathion residues can be photodegraded in soil (Smith et al. 1978; Burkhard and Guth 1979) and water (Dejonckheere and Kips 1974). Vorkamp et al. (2002) have shown that thermophilic digestion has a higher potential for methidathion degradation. Getzin (1970) reported that methidathion could be degraded rapidly in non-fumigated soils and slowly in fumigated soils, which suggests that microorganisms are primarily responsible for the degradation of methidathion. Gonzalez-Lopez et al. (1992) attributed the increase in microbial activities in agricultural soil after application of the insecticide methidathion to the fact that local soil microflora can utilize methidathion as a nutrient. Based on the relationship between dihydroartemisinin (DHA) and insecticide degradation in both sterile and non-sterile soils, Sanchez et al. (2003) proposed that microbial activity might contribute to methidathion degradation . However, most research has been under laboratory conditions, with few data showing degradation by microorganism under real field conditions.

Here, we report the isolation and identification of a bacterial strain capable of utilizing methidathion from contaminated soil as the sole carbon source. Experiments were also conducted to evaluate the potential of this strain to remove and detoxify methidathion residues in pure cultures using an orthogonal design, and its application under real field conditions in order to establish a feasible method for methidathion abatement in contaminated environments.

Materials and methods

Soils and chemicals

Soil samples were collected from an agricultural field in Longquanyi district of Chengdu city (China). This field has been used for the cultivation of peaches, and has been widely and frequently exposed to methidathion from 2002 to 2012. The contaminated soil samples taken from the top layer (0–10 cm) were sieved to remove stones and debris. Methidathion standard (99.0 %, purity) was obtained from Environmental Protection Institute of Ministry of Agriculture. HPLC-grade hexane, toluene and other reagents were purchased from Kelong Chemical Reagent Factory (Chengdu, Sichuan Province, China).

Enrichment and isolation of microorganism

The mineral salt medium (MSM) used had the following composition (per liter) : 1.0 g NH_4NO_3, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.5 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4, 0.5 g NaCl, 1.5 g K_2HPO_4 and a trace element solution 10 mL, pH 7.0. Solid medium plates were prepared by adding 1.5 % w/v agar to liquid medium. Methidathion was introduced in the form of methanol solution to give a final concentration of 50 mg/L. After 24 h of shaking and solvent evaporation, contaminated soil samples (5 g) were placed in an Erlenmeyer flask (250 mL) containing 100 mL MSM medium supplemented with methidathion as the sole source of carbon and energy. Flasks were then incubated at 30 °C on a rotary shaker (200 rpm) for 7 days and shielded from the light to avoid photodegradation of methidathion. After this time, 5 mL (5 %) of enrichment culture was transferred into 100 mL fresh MSM medium with the higher concentration of methidathion at 100 mg/L and incubated for a further 7 days under the same conditions. All MSM as mentioned above was autoclaved and cooled before use.

After the sixth transfer, bacterial strains were isolated by serially plating ten-fold dilutions of enrichment medium onto MSM agar containing 50 mg/L methidathion. After incubation at 35 °C for 3 days, different bacterial colonies were selected and further purified using the plate streaking method. Based on its higher methidathion-degradation efficiency, one pure bacterium, named strain SPL-2, was reserved for further investigation.

Strain identification

Strain SPL-2 was identified according to *Bergey's Manual of Determinative Bacteriology* (Holt et al. 1994). The 16S rRNA gene was amplified by PCR using forward primer 27F and reverse primer 1492 R (27F: 5′-AGAGTTTGATCCTGGCT CAG-3′ and 1492R: 5′- GGTTACCTTGTTACGACTT-3′). The 50 μL PCR mixtures contained 5 μL 10×PCR buffer (Mg^{2+} plus), 4 μL dNTP (2.5 mM each), 1 μL of each primer, 1 μL DNA and 0.5 μL *Taq* DNA polymerase (TaKaRa, Dalian China). PCR reactions were carried out under the following conditions: 2 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C; and then an additional 10 min cycle at 72 °C. PCR products were purified by agarose gel electrophoresis and ligated into pMD 19-T vector (TaKaRa, Dalian, China), then sequenced (Invitrogen Shanghai, China). Sequences were aligned using BLAST in NCBI (National Center for Biotechnology Information databases, http://www.ncbi.nlm.nih.gov/). A phylogenetic tree was deduced

by the neighbor-joining method (Saitou and Nei 1987) in MEGA version 4.0 (Kumar et al. 2001). The reliability of this tree was assessed by 1,000 bootstrap replicates.

Removal of methidathion by strain SPL-2 in liquid culture

Following cultivation in LB medium (yeast extract 5 g/L, tryptone 10 g/L and NaCl 10 g/L, pH 7.0) for 14 h, strain SPL-2 was centrifuged at 10,000 *g* for 8 min and then washed twice with 0.9 % (w/v) sterile NaCl solution. After adjusting the optical cell density at 600 nm (OD_{600}) to 1.2, an inoculum (5 %, v/v) was added to sterile 150 mL Erlenmeyers flasks containing 50 mL MSM with methidathion (50 mg/L). To confirm the effect of the initial concentration of methidathion on degradation, MSM medium with methidathion at concentrations of 50, 100, 150 and 250 mg/L was used. All samples were incubated under aerobic conditions on a constant temperature shaker (SUKUN, Shanghai, China) for 7 days. At time intervals of 0, 1, 3, 5, and 7 days, samples were collected from the cultures and the concentration of the selected insecticide was determined by GC-MS. Bacterial growth at the concentration of 50 mg/L was recorded by measuring OD_{600} with a UV/vis spectrophotometer (VARIAN, SpectrAA-220Fs). Each treatment was analyzed in triplicate and a blank control without microorganism was carried out under the same conditions.

Orthogonal experimental design

Instead of testing all possible combinations, the orthogonal design method tests typical pairs of combinations of factors and identifies the best combination, thus saving time and resources. For this study, we determined biodegradation capacity as the quality characteristic to be optimized and identify the control factors and their alternative levels as shown in Table 1. According to the orthogonal design method, we chose an L_9 (3^4) orthogonal array to design the orthogonal experiment as shown in Table 2. For degradation experiments, bacterial inoculum was inoculated into sterile 150 mL Erlenmeyers flasks containing 50 mL MSM and incubated under aerobic conditions on a constant temperature shaker (SUKUN) for 7 days. Each experimental group designed in Table 2 was performed in three replicates.

Table 1 Orthogonal experimental method parameters and their levels

Factor	Designation	Level 1	Level 2	Level 3
pH	A	6	7	8
Temperature (°C)	B	20	30	40
Inoculum size (OD_{600}=1.2)	C	1 %	5 %	10 %
Aeration rate (rpm)	D	100	150	200

Table 2 Design matrix and the results of methidathion biodegradation based on the orthogonal design form [L_9 (3^4)]. Levels are listed in Table 1

Experiment number	Operating factors and their levels[a]				Methidathion biodegradation rate (%)
	A	B	C	D	
1	1	1	1	1	8.74
2	1	2	2	2	63.76
3	1	3	3	3	77.99
4	2	1	2	3	19.17
5	2	2	3	1	92.97
6	2	3	1	2	40.28
7	3	1	3	2	23.87
8	3	2	1	3	48.26
9	3	3	2	1	24.78

[a] *A* pH, *B* temperature, *C* inoculum size, *D* aeration rate

Field trials and sampling

To explore the possibility of applying strain SPL-2 to bioremediate methidathion-contaminated agricultural products, field experiments were conducted on a farm in Longquanyi district of Chengdu City in China. Haricot beans and peaches were grown in two 667 m^2 open fields, respectively. To ensure all samples received the same initial concentration, we verified that no methidathion residue was detected in both experimental fields before application of this insecticide. Methidathion 40 % EC was then sprayed evenly in June 2011 using a knapsack sprayer at the rate of 2,000 L/ha with 0.8 kg/ha of active ingredient, corresponding to the recommended dosage on the label. After 2 days of controlling crop pests by methidathion, strain SPL-2 fermenting liquids at different concentrations (OD_{600}=0.2, OD_{600}=0.4) were applied onto the crops at the rate of 900 L/ha. Methidathion and strain SPL-2 fermenting liquids were both sprayed at 3:00–5:00 p.m. During the experiment, the weather was cool, without strong sunlight or rainstorm. A sample receiving no bacterial treatment was used as a control. After application of prepared SPL-2 agent, samples from both peaches and haricot beans were collected at 2, 4, 6 and 8 days for the determination of methidathion residues. All samples were transported immediately to the laboratory and stored at −20 °C in sealed polyethylene bag until extraction.

Chemical analysis

To extract methidathion from MSM, 1 mL samples collected from the medium was mixed with 2 mL hexane-toluene (1:1, v:v) in a tube capped with a Teflon screw-cap and vortexed for 30 s. The sample was then extracted in a microwave oven for 100 s at 300 W (Sanchez et al. 2000). After cooling, the organic

layer was dried over anhydrous Na_2SO_4 and then removed by a stream of nitrogen at room temperature. The residues were dissolved in 1 mL acetone for determination by GC-MS.

For methidathion extraction from agricultural products, samples (15 g) were weighed accurately, chopped and extracted with 60 mL extraction solution (hexane: acetone, 50:50 by volume) in a PTFE-lined extraction vessel, which was shaken vigorously for 30 min and then treated with by ultrasound for 5 min. Subsequently, the mixture was extracted for 9 min in a microwave oven at 475 W (Bouaid et al. 2000). After extraction, the vessel was cooled to room temperature before being opened. After filtering, the residue was rinsed with 20 mL solvent and combined with the supernatant. Finally, the extract was evaporated to dryness using a vacuum rotary evaporator and dissolved in 5 mL acetone for GC-MS analysis. According to the extraction procedure described above, average recoveries of methidathion in MSM ranged from 93.5 % to 106.8 %, and on the two crops extraction values were 90.7 % to 103.3 %.

Methidathion determination

Methidathion residues, extracted as described above from salt media or from crop samples, were analyzed using a GC-MS device (SHIMADZU, Japan) equipped with a RTX-5 MS capillary column (30 m×0.25 mm×0.25 μm). The operating conditions were as follows: initial oven temperature 60 °C with 2 min, heating at 20 °C/min to 180 °C for 1 min, and then at 10 °C/min to 265 °C for 2 min. An injector temperature of 250 °C was used. A 1 μL volume of extract was injected in the splitless mode with helium (99.999 %) as the carrier gas at 1 mL/min. A mass spectrometer was operated in the electron impact positive ion (EI+) mode with a source temperature of 200 °C and the electron energy was 70 eV. The insecticide was quantified by automatic integration of the peak area. Certified standards of methidathion were used for external calibration.

Data analysis

The data of the first-order kinetics model was calculated using Excel (Microsoft, Seattle, WA). Statistical Package for the Social Sciences (SPSS, Chicago, IL) 16 statistical software was used for range analysis and the analysis of variance (ANOVA) of the data in the orthogonal experimental design.

Results and discussion

Strain identification

Afer about 1 month of enrichment cultivation, a methidathion-degrading strain, named SPL-2, was isolated from a mixture of collected soil samples contaminated with methidathion for many years. Strain SPL-2 forms red, convex, opaque and irregular morphological colonies on LB solid medium. It was found to be a Gram-negative, nonspore forming, rod-shaped bacterium with flagella, with a size of 0.7–0.9 μm× 1.0–1.2 μm. The strain utilized α-D-glucose, D-galactose, D-fructose, maltose, and D-sorbitol, and also reduced nitrate to nitrite. A 1,446-bp sequence of the 16S rRNA of SPL-2 was determined (GenBank accession NO.JN400353). 16S rRNA sequence alignment indicated that strain SPL-2 was homologous with *Serratia* sp. FS014 (HM245061, 99 % similarity) and *Pseudomonas fluorescens* strain YR20 (HM224401, 99 % similarity). To identify the phylogeny of strain SPL-2, strains from different species were chosen to construct a phylogenetic tree based on 16S rRNA sequences (Fig. 1). The phylogenetic analysis revealed that strain SPL-2 clustered together with *Serratia* sp. and was positioned at the base of most *Serratia* sp. strains, relatively far from *P. fluorescens* strains. On the basis of the 16s rRNA results, morphological characteristics and biochemical tests, the strain was identified as *Serratia* sp.

According to previous studies, *Serratia* sp. are known to be very metabolically active bacteria, exhibiting efficient degradation of pollutants such as diazinon (Cycon et al. 2009), 2,4,6-trinitrotoluene (Montpas et al. 1997), dichlorodiphenyltrichloroethane (DDT) (Bidlan and Manonmani 2002), syntheticdyes (Verma and Madamwar 2003), hexachlorobutadiene (Li et al. 2008), and dibenzofuran (Jaiswal and Thakur 2007). However, there are no reports concerning its ability to metabolize methidathion.

Microbial degradation of methidathion in MSM

Figure 2 shows the growth curve of SPL-2 on methidathion and its ability to remove this insecticide from liquid culture. After incubation for 7 days, about 84.1 % of the 50 mg/L methidathion initially added to the culture was degraded by strain SPL-2 when methidathion was used as the sole carbon source. Accordingly, the OD_{600nm} measurements of the strain increased steadily in MSM.

Degradation curves of methidathion were examined at levels of 50, 100, 150 and 250 mg/L in MSM at pH 7.0 and 30 °C (Fig. 3). Degradation processes at levels of 50, 100, 150 and 250 mg/L were characterized by rate constants of 0.2522, 0.2578, 0.157, 0.0359 /day, following a classic first-order rate kinetics; the rate of methidathion degradation was 6.443, 12.886, 15.257 and 8.300 mg L^{-1} day^{-1} after 7 days of incubation, respectively. The corresponding half-lives calculated from the first-order function were 2.75, 2.69, 4.41 and 19.30 days, respectively (Table 3). No significant change in methidathion concentration was observed in any of the sterilized control bottles. As shown in Fig. 3 and Table 3, higher concentrations of methidathion (250 mg/L) resulted in a lower

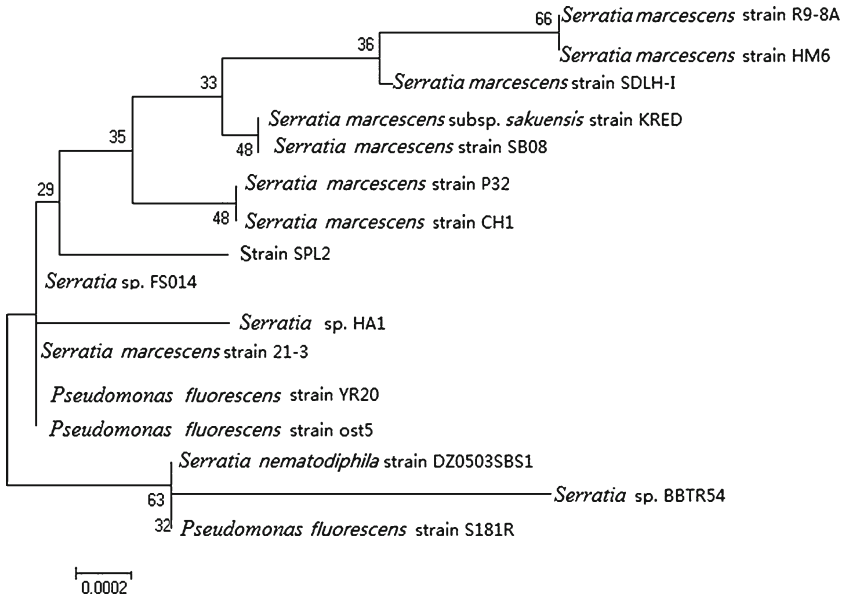

Fig. 1 Phylogenetic tree of strain SPL-2 and related species based on 16S rRNA gene sequences

degradation rate, suggesting a possible toxic effect of this compound on the strain. A similar phenomenon, showing an obvious inhibitory effect on metabolization capability, has been found in other organophosphorus pesticides (Liang et al. 2009). Wang et al. (2006) also reported that the strain *Bacillus laterosporus* DSP showed high degrading ability of chlorpyrifos (1 and 10 mg/L), but concentrations of chlorpyrifos up to 100 mg/L exhibited an obvious inhibitory effect on the degradation capacity of the strain. It has been reported that several bacterial species, such as *Providencia* sp. (Rani et al. 2008), *Burkholderia* sp. (Hong et al. 2007), *Pseudomonas* sp. (Cycon et al. 2009), *Klebsiella* sp. (Ghanem et al. 2007), can utilize organophosphate insecticides as sole carbon sources. However, little is known about the degradation of methidathion residues by strains in pure cultures.

Factors affecting methidathion biodegradation and optimization by orthogonal design

Results and range analysis of orthogonal experimental design

The efficiency of methidathion biodegradation is affected by environmental factors known to limit the activity of strain SPL-2. Thus, there is a need to identify key factors and optimal conditions so as to make the bioremediation process more effective. We conducted nine experiments using an orthogonal experimental design L_9 (3^4) to determine the optimal biodegradation conditions for the methidathion-degrader strain SPL-2. The results of the orthogonal experimental design are presented in Table 2. Changes in methidathion concentration in the autoclaved culture medium without bacterial inoculation can be neglected. According to the results listed in Table 2, the methidathion degradation rate was maximal at the following combination of four factors: pH 7, temperature 30 °C, inoculum size 10 %, aeration rate 100 rpm. Under these conditions, in experiment 5 the degradation rate reached 92.97 %. In contrast, the lowest methidathion biodegradation rate (~8.74 %) was obtained in experiment 1. The results of range analysis are presented in Table 4. K_1, K_2 and K_3 were the average values of the results of each level in the orthogonal method experiment, and the optimal level of each variable can be determined by comparing the value of K_i (i=1, 2, 3). Range=max [K_1, K_2, K_3]−min [K_1, K_2, K_3]. Based on the principle of "The larger the better," range analysis indicates the best levels for each single variable and estimates which variable is most important for the biodegradation process. The results are listed in Table 5.

Analysis of variance

A statistical ANOVA was applied to estimate which process factors significantly affect process responses. We conducted the F test (the Fischer ratio), which is a good tool for deciding

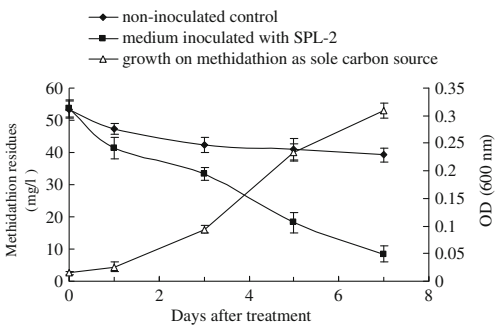

Fig. 2 Degradation of methidathion by *Serratia* sp. SPL-2 in mineral salt medium (MSM). *Error bars* Standard deviation of three replicates

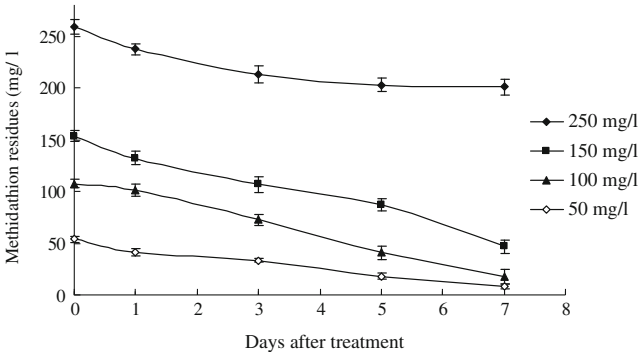

Fig. 3 Effect of methidathion concentration on its biodegradation in MSM. *Error bars* Standard deviation of three replicates

which process factor has a significant effect on degradation rate. The F value for each factor is a ratio of the mean of the squared deviations to the mean of the squared error. An F value was calculated from the process results and compared to the F critical value (critical values for the Fischer ratio), which can be found in most statistics textbooks. If the F value calculated in this study is larger than the F critical value (19.000), it indicates that the statistical test is significant at the confidence level selected (P=0.05). If not, we consider that the statistical test is not significant at this level. Table 6 shows the ANOVA results for orthogonal experimental design based on the valves of biodegradation rate at the end of the 7-day experiment. The calculated F values shown in Table 6 suggest that temperature had the most significant impact on degradation rate, followed by inoculum size, while pH value and aeration rate had only little effect on methidathion biodegradation. This means that initial temperature and inoculum size play an important role in pesticide degradation and should be given prior consideration when planning biotreatment processes of contaminates. Accordingly, the optimal conditions for methidathion biodegradation by strain SPL-2 in pure cultures was 30 °C, an inoculum size of 10 % (OD_{600}=1.2), pH= 7 and an aeration rate of 200 rpm.

Preliminary information related to the environmental factors affecting efficiency of pollutant degradation by bacteria has been reported by many researchers. Temperature fluctuation seems to be the most important factor. Li et al. (2008) revealed that the optimal incubation temperature for HL1 to degrade HCBD in pure cultures was 30 °C, and that growth of strain HL1 was adversely affected when incubated at 10 and 40 °C. Biodegradation experiments carried out with *Serratia* sp. revealed that a temperature of 38 °C enhanced the biodegradation rate of etheramine EDA 3B (Araujo et al. 2010). Temperature can double microbial metabolism rate for each 10 °C between 10 and 40 °C (Leahy and Colwell 1990). Kyriakidis et al. (2000) found that the half-lives at 15 °C of orange and peach juice are 28 and 30 times longer than the corresponding half-lives at 40 °C, which means that higher storage temperature might contribute to methidathion degradation. Similarly, the effect of temperature on methidathion degradation by *Serratia* sp. was also significant, with degradation rates being much higher at 30 °C and 40 °C than the rate at 20 °C listed in Table 4. On the other hand, the degradation rate at 30 °C was higher than that at 40 °C, suggesting that strain SPL-2 might play an important role in the degradation process.

Inoculum size has been suggested as another important factor affecting pesticide biodegradation efficiency by microbes, and low inocula amount in shake flasks may reduce the number of active degrading bacteria to an insufficient level. A positive correlation was noted between inoculum size and the aerobic degradation rate of dichlorodiphenyltrichloroethane (DDT) by strain *Serratia* sp. (Bidlan and Manonmani 2002). Similarly, the rates of methidathion metabolism were found to increase when inoculum size increased from 1 % to 10 %. However, the effect of inoculum size on biodegradation of beta-cypermethrin was insignificant, probably because the range tested was narrow (from 0.1 to 0.3) (Zhang et al. 2010).

Table 3 Degradation rate constant (k) and rate of methidathion degradation (v) by SPL-2 in mineral salt medium

Methidathion concentration (mg/L)	Rate of methidathion degradation (mg L^{-1} day^{-1})	Degradation rate constant (day^{-1})	DT_{50} (day)	R^2
50	6.443	0.2522	2.75	0.9597
100	12.886	0.2578	2.69	0.9441
150	15.257	0.157	4.41	0.9338
250	8.3	0.0359	19.30	0.8826

Table 4 Range analysis for the L_9 (3^4) orthogonal array experiment. Levels are listed in Table 1

K value	Methidathion degradation rate (%)			
	A[a]	B	C	D
1	50.163	17.260	32.427	42.163
2	50.807	68.330	35.903	42.637
3	32.303	47.683	64.943	48.473
Range	18.504	51.070	32.516	6.316

[a] *A* pH, *B* temperature, *C* inoculum size, *D* aeration rate

Table 5 Optimum level for each single factor in the $L_9(3^4)$ orthogonal array experiment. Levels are listed in Table 1

Factor[a]	Optimal level
A	2
B	2
C	3
D	3

[a] *A* pH, *B* temperature, *C* inoculum size, *D* aeration rate

Table 6 ANOVA results of degradation rate in the $L_9(3^4)$ orthogonal array experiment. Levels are listed in Table 1. *SSD* Sum of squares of deviations

Methidathion degradation rate (%)				
Operating factor	dof	SSD	F ratio	Fcr critical value[a]
pH	2	661.767	8.930	19.000
Temperature	2	3,960.009	53.436	19.000
Inoculum size	2	1,912.742	25.811	19.000
Aeration rate	2	74.107	1.000	19.000
Error	2	74.1		
Total	10	6,682.725		

[a] Significance level at a 95 % confidence interval, $F_{0.05}(2,2)=19$

According to Hartley and Kidd (1987), methidathion is metabolized rapidly in alkaline and strongly acidic conditions, but is rather stable in neutral and weakly acid conditions. In this study, we investigated the effect of initial pH value on methidathion degradation by SPL-2 in pure cultures with respect to its effects on strain activity; we chose pH values ranging from 6 to 8 in the orthogonal experimental design in order to exclude abiotic degradation. According to previous research, maximum (93 %) PCP degradation by *Serratia* sp. was observed at pH 7.0; on the other hand, a sharp decline (70 and 75.16 %) was recorded at pH 6.0 and 9.0, respectively (Singh et al. 2009). More than 90 % decolorization of RFB and PBB-HGR was obtained by *Serratia marcescens* at pH 7.0 (Verma and Madamwar 2003). Complete HCBD removal by strain *Serratia marcescens* HL1 was observed on day 7 when pH values changed from 7.0 to 8.0 (Li et al. 2008). These data and our results suggest that strain *Serratia* sp., identified in the present study, can be adapted for neutral cultures.

Variations in aeration rate did not favor methidathion degradation according to the results of orthogonal experimental design. Likewise, aeration rates between 50 rpm and 200 rpm had little effect on PCP degradation of *Serratia marcescens* (Singh et al. 2007), while it was also observed that very high aeration rates might adversely affect biodegradation due to bacterial cell damage.

Kinetics of methidathion degradation on agricultural products

Numerous models have been used to describe the biodegradation process of many organic pollutants, and first-order kinetics have been used frequently to describe this course (Feng et al. 2004; Xu et al. 2005; Tao et al. 2008). Similarly, we found that the disappearance of methidathion on agricultural products follows the first-order kinetic model according to the results found in this field trial. A first-order kinetics model could be constructed by logarithmic transformation, with the form:

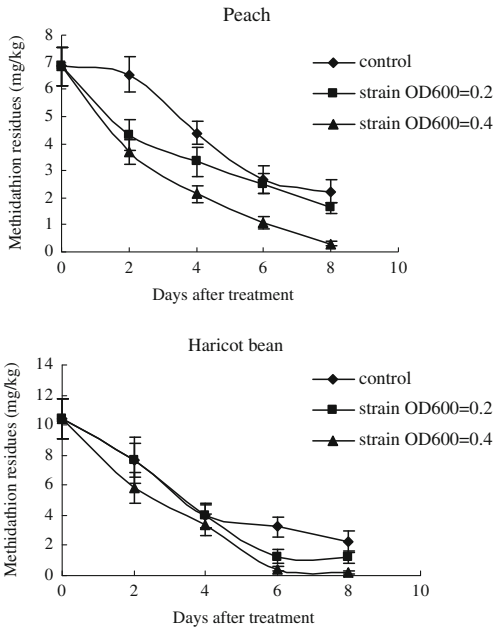

Fig. 4 Degradation curves of methidathion in various treatments on peach and haricot bean. *Error bars* Standard deviation of three replicates

$$\ln C = -k\,t + A$$

Where C is the concentration of methidathion residues at time t, K is the first-order kinetic constant (day^{-1}), t is the degradation period in days, and A is the constant. The half-life ($t_{1/2}$) was determined from the k value for each experiment, according to the equation $t_{1/2} = \log 2/k$.

Degradation processes of methidathion in various treatments on agricultural products under field conditions are presented in Fig. 4. The first-order kinetic model data for methidathion degradation are summarized in Table 7. From these results, half-lives of methidathion on control haricot beans and peaches not inoculated with strain SPL-2 were measured to be 3.53 and 4.40 days, while they were 2.27 and 4.04 days in the treatment with $OD_{600}=0.2$, and 1.33 and 1.87 days in the treatment with $OD_{600}=0.4$. In contrast

Table 7 First-order kinetic data of methidathion degradation in field trials

Sample	Treatment	DT_{50} (day)[a]	Average V (mg kg^{-1} day^{-1})	R^2 [b]
Peach	control	4.40	0.58	0.9485
	$OD_{600}=0.2$	4.04	0.655	0.989
	$OD_{600}=0.4$	1.87	0.81875	0.9693
Haricot bean	control	3.53	1.02375	0.9738
	$OD_{600}=0.2$	2.27	1.1525	0.9353
	$OD_{600}=0.4$	1.33	1.28	0.9432

[a] Half-lives of degradation for methidathion DT_{50} values on crops were obtained by the function of $DT_{50}=\ln 2/k$

[b] Coefficients of determination R^2 were obtained using Microsoft Excel.

to the controls, the DT_{50} values of methidathion were shortened by 35.7 %, 8.2 % and by 62.3 %, 57.5 % on OD_{600}= 0.2 and OD_{600}=0.4 treated haricot beans and peaches, respectively. Accordingly, in the case of crops uninoculated with strain SPL-2, peaches and haricot beans have lower average rate of methidathion degradation of 0.5800 and 1.0237 mg kg^{-1} day^{-1}. After 8 days of experiment, 76.38 % and 88.31 % of the applied dose of methidathion on peaches and haricot beans were degraded at a strain concentration of OD_{600}=0.2, and the corresponding values at strain concentration OD_{600}=0.4 were 95.48 % and 98.08 %, which gave final residual values of methidathion of 0.31 and 0.20 mg/kg, respectively. The results showed that higher concentration strain inoculum (OD_{600}=0.4) degraded methidathion on peaches and haricot beans more efficiently than lower concentration strain inoculum (OD_{600}=0.2) and blank control sets. The lower concentration strain inoculum sets (OD_{600}=0.2) exhibited a lower degradation rate, which could be due to only a small part of introduced strain being able to survive and take part in pesticide degradation (Ramadan et al. 1990). Obviously, the degradation rate of methidathion residues on the two crops differ, which may depend largely on the adsorbed amounts of bacterial agent on the crop surface. Moreover, many abiotic factors, including pesticide formulation, temperature, humidity, solar radiations and precipitation, might have an effect on bacterial activity.

Conclusion

An enrichment procedure was applied successfully to isolate a single bacterial strain identified as *Serratia* sp. that can participate in efficient degradation of the organophosphorus insecticide methidathion. Orthogonal experimental design L_9 (3^4) established the optimum conditions for methidathion biodegradation by strain SPL-2 in pure culture. Range analysis and ANOVA results indicate that temperature and inoculum size were statistically inferred to have significant influences on degradation rate. Degradation kinetics analysis showed that the degradation process of methidathion by the bacterial strain SPL-2 on peaches and haricot beans fit well with a first-order kinetics model, and the results suggested that microbial degradation might be a promising way for the removal or detoxification of methidathion pesticide on vegetable and fruit. However, more investigations will be required to elucidate the pathway used by strain SPL-2 to metabolize methidathion. There is also a need for further research into the enzymatic and genetic aspects of methidathion degradation by the isolated bacteria.

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