Biodegradation (2006) 17: 487–494
DOI 10.1007/s10532-005-9020-z

Characterization of a fungal strain capable of degrading chlorpyrifos and its use in detoxification of the insecticide on vegetables

Yun Long Yu[1,*], Hua Fang[1], Xiao Wang[1], Xiao Mao Wu[1], Min Shan[1] & Jing Quan Yu[2]
[1]*Department of Plant Protection, College of Agriculture & Biotechnology, Zhejiang University, 310029, Hangzhou, Zhejiang, People's Republic of China;* [2]*Department of Horticulture, College of Agriculture & Biotechnology, Zhejiang University, 310029, Hangzhou, Zhejiang, People's Republic of China (*author for correspondence: e-mail: ylyu@zju.edu.cn)*

Accepted 7 September 2005

Key words: biodegradation, cell-free extract, chlorpyrifos, microorganism, residue, vegetable

Abstract

A fungal strain capable of utilizing chlorpyrifos as sole carbon and energy sources was isolated from soil by enrichment cultivation approach. The half-lives of degradation (DT_{50}) for chlorpyrifos at concentrations of 1, 10, and 100 mg l^{-1} by the fungal strain DSP in mineral salt medium were measured to be 2.03, 2.93, and 3.49 days, respectively. Two cell-free extracts [E (1:10) and E (1:20)] from the fungal strain DSP in bran–glucose medium were prepared and used to enhance chlorpyrifos degradation on vegetables. Compared with the controls, the DT_{50} of chlorpyrifos were reduced by 70.3%, 65.6%, 80.6%, 80.6%, and 86.1%, and by 53.8%, 43.2%, 66.0%, 54.3%, and 67.7% on E (1:20) and E (1:10) treated pakchoi, water spinach, Malabar spinach, haricot beans, and pepper, respectively. The 7-day residual values (R_7) of chlorpyrifos on E (1:10) treated vegetables were all lower than the corresponding maximum residue levels of European Union (EU MRLs), except that the R_7 value on haricot beans was slightly higher than the corresponding EU MRLs. The results indicate that cell-free extracts could rapidly degrade chlorpyrifos residues on vegetables.

Introduction

Greenhouse vegetable production has been developed rapidly in China since 1980s and has become a main source of vegetable supply. However, the warm and humid environment in a greenhouse provides favorable conditions for fast reproduction of insect pests and disease causal pathogens, which in turn result in excessive application of pesticides (van Lenteren 2000). On the other hand, some insect pests and pathogens may also develop resistance or tolerance to the conventionally used pesticides due to their single mode of action and this leads to frequent application of pesticides at higher dosages (Gajbhiye 2004). Furthermore, pesticides often dissipate slower on greenhouse vegetables due to the hermetic environments in greenhouses (Aguilera-del Real et al. 1999; Garau et al. 2002; Hernandez Torres et al. 2002; Martinez Galera et al. 2003). The excessive and frequent application of pesticides may result in high levels of pesticide residues accumulated on vegetable products, which poses a potential health hazard to consumers (Bolognesi & Morasso 2000; Fan & Jackson 1989; Tweedy et al. 1991). Therefore, there is an increasing concern regarding rapid elimination of pesticide residues on vegetables.

Chlorpyrifos [*O*,*O*-diethyl-*O*-(3,5,6-trichloro-2-pyridinyl)phosphorothionate] is a broad spectrum organophosphorus insecticide and acaricide and is widely used for insect pest control on grain, cotton, fruit, and vegetable crops in China. Due to

its high acute toxicity, chlorpyrifos may affect the central nervous system, the cardiovascular system, and the respiratory system as well as cause skin and eye irritation (Oliver et al. 2000; Serrano et al. 1997). The widespread use of chlorpyrifos in agriculture has increased the public concern on potential human health risks that may result from acute or chronic dietary exposure to chlorpyrifos residues in food (Cochran et al. 1995; Martínez Vidal et al. 1998). The use of microbial cell-free extracts might be a promising way to rapidly detoxify chlorpyrifos residues. The degradation of chlorpyrifos by cell-free extracts in pure cultures and soils has been well investigated (Brown 1980; Chen & Mulchandani 1998; Dave et al. 1993; Horne et al. 2002; Mallick et al. 1999; Singh et al. 2004). However, little is known on degradation of chlorpyrifos residues by cell-free extracts on vegetables under agricultural conditions.

In the present study, we report isolation and identification of a fungal strain capable of utilizing chlorpyrifos as sole carbon and energy sources from soil and degradation of chlorpyrifos in pure cultures and on vegetables by this fungal strain and its cell-free extract. The objectives of this study were to test the capability of the isolated fungal strain and its cell-free extract to detoxify chlorpyrifos residues under experimental conditions, and to explore the feasibility of using a cell-free extract to detoxify chlorpyrifos residues on vegetables under agricultural conditions.

Materials and methods

Chemicals

Chlorpyrifos standard (≥99.5% purity) was obtained from Institute for the Control of Agrochemicals, Ministry of Agriculture, China. Chlorpyrifos 40% EC was obtained from Xinnong Chemicals Co., Zhejiang, China. Petroleum ether (60–90 °C) was purchased from Fangting Chemicals Co., Zhejiang, China. All solvents were of analytical reagent grade and were redistilled in a full glass system before use.

Isolation of microorganism

Soil samples from farm soil, tree rhizosphere soil, sediment of a sewer, sludge, and piggery soil were collected from Huajiachi Campus, Zhejiang University, Hangzhou, China. These five samples were homogenized and divided into three subsamples (50 g). Each subsample was diluted with 100 ml of sterilized water in a 250 ml Erlenmeyer flask. A 1 ml aliquot of soil supernatant was used as inoculant into a 100 ml Erlenmeyer flask containing 20 ml of sterile mineral salt medium ($MgSO_4{\cdot}7H_2O$, 0.40 g; $FeSO_4{\cdot}7H_2O$, 0.002 g; K_2HPO_4, 0.20 g; $(NH_4)_2SO_4$, 0.20 g; $CaSO_4$, 0.08 g; H_2O, 1000 ml; pH 7.2) supplemented with 50 mg l^{-1} of chlorpyrifos as the sole source of carbon and energy. After incubation for 1 week at 30 °C on a rotary shaker (150 rpm), the culture was inoculated into 20 ml of sterile mineral salt medium with chlorpyrifos (100 mg l^{-1}) and incubated for another 1 week under same conditions. The cultures were repetitively acclimated in sterile mineral salt medium with increasing concentrations of chlorpyrifos, ranging from 100 to 200 mg l^{-1}. Subsequently, the cultures were cultivated onto potato–dextrose–agar (PDA) plates (potato, 200 g; dextrose, 20 g; agar, 20 g; chlorpyrifos, 0.1 g; H_2O, 1000 ml; pH 7.2) and incubated for 3 days at 30 °C. Chlorpyrifos-degrading fungal strain DSP was isolated from colonies formed on the plates.

Identification of microorganism

The isolated fungal strain DSP was purified by repetitive subculturing on PDA plates with chlorpyrifos (100 mg l^{-1}). Identification of the DSP strain was performed according to its morphological characteristics (Barnett & Hunter 1972; Dai 1987). For further characterization, 18S rDNA gene sequences of the DSP strain were determined (Ahren et al. 1998). Identification of the fungal strain DSP was carried out by Zhejiang Microbiology Institute, Hangzhou, China.

DNA extraction and purification

The fungal strain DSP was cultivated for 2 weeks on PDA plates at 25 °C. The mycelia were scraped, collected and used for the genomic DNA extraction. Genomic DNA was extracted according to standard CTAB-based protocols (Lee & Taylor 1990; White et al. 1990), purified by Clonetech tissue kit and stored at −20 °C.

18S rDNA amplification and sequencing

The 18S rDNA was amplified using the polymerase chain reaction (PCR) with genomic DNA as template and a pair of universal primers, NS1 (5′-GTAGTCATATGCTTGTCTC-3′) and NS8 (5′-TCCGCAGGTTCACCTA-3′). The PCR products were purified using QIAGEN PCR Purification kit (Qiagen) and sequenced by the dideoxy chain termination method on a model 373 automated DNA sequencer (Applied Biosystems). Sequence data were compared with other fungal 18S rDNA gene sequences in the GenBank database by Blast searching and deposited in the NCBI GeneBank database.

Inoculum preparation

The fungal strain DSP was cultivated in 250 ml Erlenmeyer flasks containing 200 ml of Luria–Bertani (LB) medium (peptone, 10 g; beef extract, 5 g; NaCl, 5 g; chlorpyrifos, 0.1 g; H_2O, 1000 ml; pH 7.2) at 30 °C on a rotary shaker (150 rpm). After 5 days, the culture was collected by filtration, washed thrice with 20 ml of NaH_2PO_4–Na_2HPO_4 buffer (0.1 mol l^{-1}, pH 7.0), and then suspended in the same buffer. These mycelia preparations were used as inoculants.

Microbial degradation of chlorpyrifos in mineral salt medium

Chlorpyrifos was added to a 100 ml Erlenmeyer flask containing 20 ml of mineral salt medium to final concentrations of 1, 10, 100 mg l^{-1}, respectively. Fifty milligrams of mycelia (wet weight) was inoculated into the mineral salt medium and incubated at 30 °C on a shaker (150 rpm). At the time intervals of 0, 1, 3, 5, and 7 days, the whole culture was sampled for the assay of chlorpyrifos. Degradation of chlorpyrifos in mineral salt medium without inoculation of fungal mycelia was used as control. All experiments were triplicated.

Cell-free extracts

The fungal strain DSP was inoculated into 250 ml Erlenmeyer flasks containing 200 ml of bran–glucose medium (glucose, 5 g; bran, 5 g; NaH_2PO_4, 3.5 g; K_2HPO_4, 5 g; $(NH_4)_2SO_4$, 2.5 g; $MgSO_4$, 0.2 g; NaCl, 0.1 g; water, 1000 ml; pH 7.2) and incubated for 5 days at 30 °C on a shaker at 150 rpm. The cell-free extract was obtained by disrupting medium containing hyphae through High-Pressure Cells Press JG-1A (Ningbo Xinzhi Biology Technology Co., China) at 15 MPa. Two cell-free extracts were prepared by diluting the cell-free extract at 1:10 and 1:20 with NaH_2PO_4–Na_2HPO_4 buffer (0.1 mol l^{-1}, pH 7.0), viz., E (1:10) and E (1:20). All cell-free extracts were stored at 4 °C until use.

Field trial and sampling

The experiments were conducted in a greenhouse and open field located on the farm of Huajiachi Campus, Zhejiang University, China. The greenhouse was constructed of polyethylene with a flat roof and a lateral window (25 m × 0.5 m) and covered with fine netting. The greenhouse and open field were 90 and 60 m^2, respectively.

Malabar spinach (*Basella rubra* L.), haricot beans (*Phaselous vulgaris* L.) and pepper (*Capsicum frutescens* L.) were grown in 30 m^2 plots in a greenhouse, respectively. Pakchoi (*Brassica Chinensis* L.) and water spinach (*Ipomoea aquatica* Forsk) were cultivated in 30 m^2 plots in an open field, respectively. Chlorpyrifos 40% EC was applied at the rate of 2500 l ha^{-1} with 0.84 kg ha^{-1} of active ingredient, corresponding to double dosage of the recommendation dosage. After one day, two cell-free extracts were sprayed onto the crops at the rate of 375 l ha^{-1}. The experiments were carried out in autumn 2004.

After application of chlorpyrifos, samples were collected at 0, 1, 3, 5, 7, and 10 days, respectively, from five vegetable crops. These samples were chopped and divided into three subsamples (25 g), which were stored in individual polyethylene bag at −20 °C until extraction.

Extraction of chlorpyrifos in mineral salt medium and on vegetables

To determine residual chlorpyrifos concentrations in DSP cultures, the culture was transferred to a 250 ml separating funnel, and extracted thrice with 30 ml of dichloromethane for each. The organic phase was dried over anhydrous sodium sulphate and collected in a 250 ml flat-bottom flask, followed by concentration to almost dryness with a

slight N_2 stream. The residue was redissolved with 5 ml of redistilled petroleum ether prior to determination. To measure chlorpyrifos adsorbed by the fungal strain DSP, the mycelia of DSP were collected by centrifugation and homogenized (10,000 rpm) with 20 ml of petroleum ether in a blender for 2 min. The mixture was extracted as described above.

Vegetable samples (25 g) and anhydrous sodium sulfate of 80 g were homogenized (10,000 rpm) in a blender with 80 ml of petroleum ether for 2 min. The mixture was decanted and filtered through a 7 cm buchner funnel. The filter cake was washed thrice with 25 ml of redistilled petroleum ether. The extracts were collected in a 250 ml flat-bottom flask, concentrated to almost dryness with a slight N_2 stream, and diluted to 5 ml with redistilled petroleum ether for the determination by GC.

Gas chromatography analysis

Chlorpyrifos was assayed by gas chromatography GC-9790 (Fuli Analysis Apparatus Co., China) equipped with FPD detector. A fused silica capillary column (Pesticide Residue-II, 30 m $\times$ 0.32 mm $\times$ 0.25 μm) (Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, China) was employed. The temperatures of injection port, column, and detector were 240, 230, and 250 °C, respectively. The flow of gas was as follows: nitrogen, 50 ml min^{-1}; air, 80 ml min^{-1}; hydrogen, 120 ml min^{-1}.

Fortified recovery study

A recovery study was carried out at four spiking levels, 0.1, 1, 10, and 100 mg l^{-1} in mineral salt medium and 0.01, 0.1, 1, and 10 mg kg^{-1} in five vegetables that had not been treated with the pesticide. Three replicates of each recovery assay and blank sample of mineral salt medium and vegetables were extracted and analyzed as described above.

Results and discussion

Identification of microorganism

By using the enrichment cultivation approach, we isolated a fungal strain, DSP, that could utilize chlorpyrifos as sole carbon and energy sources. The fungal strain DSP appeared as round and wooly colonies (4.5 cm in diameter), after cultivation of 5 days on PDA medium at 30 °C. The color of the colony turned from carmine to rufous from the reverse after prolonged cultivation. The mycelia were dense, massive, and radiate. The hyphae with septa and branches were white. No odor and exudation were detected. Conidiophores had an erect stipe which was ovoid- or ellipse-shaped, surrounded by slimy droplets. The fungal strain DSP was identified as an unknown species of *Verticillium* according to above-mentioned morphological characteristics by conventional method. Analysis of the 18S rDNA gene sequences (NCBI GenBank Accession No. DQ153250) also revealed that the DSP has a high level of homology (99%) to those from other *Verticillium* species.

Fortified recovery of chlorpyrifos

The average recoveries of chlorpyrifos in mineral salt medium and on five vegetables tested are summarized in Table 1. In all cases, the recoveries of chlorpyrifos ranged from 85.4% to 102.6%, with relative standard deviation (RSD) $\leq$ 6.9%. These data indicate that the extraction method is efficient for analysis of the insecticide residues.

Microbial degradation and its mechanism of chlorpyrifos

The degradation curves of chlorpyrifos at levels of 1, 10, and 100 mg l^{-1} by the fungal strain DSP in mineral salt medium are shown in Figure 1. The degradation rates for chlorpyrifos at concentrations of 1, 10, and 100 mg l^{-1} were calculated to be 0.139, 1.229, and 11.014 mg $(dl)^{-1}$ after incubation for 7 days, respectively. The degradation rates of chlorpyrifos increased almost linearly with increasing concentrations of chlorpyrifos ($r^2 = 0.9999$), suggesting that the degradation is subjected to pseudo-first-order kinetics. With the first-order kinetic function, the DT_{50} of chlorpyrifos at concentrations of 1, 10, and 100 mg l^{-1}, were calculated to be 2.03, 2.93, and 3.49 days, respectively (Table 2). In the controls, the hydrolysis percentages of chlorpyrifos were less than 5%.

The degradation products of chlorpyrifos at concentration of 100 mg l^{-1} after incubation for

Table 1. Recovery of chlorpyrifos in mineral salt medium and on vegetables

Sample	Chlorpyrifos (mg l^{-1} or mg kg^{-1})	Sample weight (g)	Recovery (%)	RSD (%)
Mineral salt	100	20	98.6	1.5
medium	10	20	96.2	5.1
	1	20	100.2	3.3
	0.1	20	97.0	4.9
	10	25	92.5	3.2
Pakchoi	1	25	94.2	3.1
	0.1	25	101.8	6.0
	0.01	25	100.4	6.5
	10	25	89	3.4
Water spinach	1	25	91.6	4.1
	0.1	25	96.4	6.9
	0.01	25	92.7	2.8
	10	25	85.6	6.7
Malabar spinach	1	25	90.2	5.2
	0.1	25	85.4	6.5
	0.01	25	93.5	1.5
	10	25	97.4	6.6
Haricot beans	1	25	95	2.2
	0.1	25	101.2	3.8
	0.01	25	96	4.0
	10	25	98.9	4.9
Pepper	1	25	98.5	3.8
	0.1	25	102.6	3.1
	0.01	25	100.4	3.5

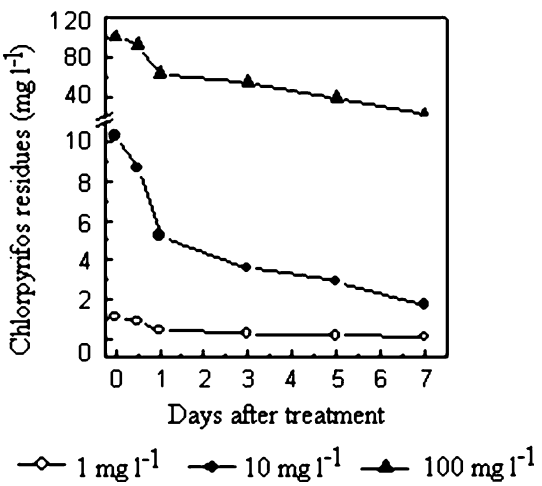

Figure 1. Degradation curves of chlorpyrifos by the fungal strain DSP.

7 days were identified with HP6890GC/HP5973MSD. Four metabolites were detected at *m/z* 129 (a), 196 (b), 198 (c), and 322 (d), respectively. Their MS spectra and the proposed structures are shown in Figure 2.

Table 2. Rate of chlorpyrifos degradation by the fungal strain DSP in mineral salt medium

Chlorpyrifos (mg l^{-1})	Total degradation rate (mg $(dl)^{-1}$)	DT_{50} (days)	r^2
1	0.139	2.03	0.9134
10	1.229	2.93	0.9328
100	11.014	3.49	0.9563

The metabolite C (3,5,6-trichloro-2-pyridinol, TCP), the major degradation product of chlorpyrifos, has been reported frequently in pure cultures (Mallick et al. 1999), water (Liu et al. 2001), vegetable (Zayed et al. 2003), fruit (Velasco-Arjona et al. 1997) and soil (Robertson et al. 1998; Singh et al. 2003; Velasco-Arjona et al. 1997). The accumulation of the products was not found by the determination after incubation for 9 days. The degradation products were similar to those observed in previous studies (Mallick et al. 1999; Racke et al. 1988; Robertson et al. 1998; Singh et al. 2003).

Degradation of chlorpyrifos by cell-free extracts on vegetables

The degradation curves of chlorpyrifos by cell-free extracts on five vegetables are shown in Figure 3. The kinetic data of chlorpyrifos degradation calculated with the first-order function are summarized in Table 3. The DT_{50} of chlorpyrifos on five vegetables ranged from 1.69 to 4.90 days in the control sets, 1.11 to 4.22 days in the treatments with E (1:20), and 0.73 to 3.32 days in the treatments with E (1:10). Compared with the controls, the DT_{50} of chlorpyrifos were reduced by 70.3%, 65.6%, 80.6%, 80.6% and 86.1%, and by 53.8%, 43.2%, 66.0%, 54.3%, and 67.7% on E (1:20) and E (1:10) treated pakchoi, water spinach, Malabar spinach, haricot beans, and pepper, respectively.

The preharvest interval of chlorpyrifos on vegetables was recommended for 7 days in China. The 7 day residual values (R_7) of chlorpyrifos on five vegetables ranged from 0.25 to 0.92 mg kg^{-1} in the controls, 0.06 to 0.56 mg kg^{-1} in treatments with E (1:20), and 0.01 to 0.43 mg kg^{-1} in treatments with E (1:10). The R_7 values of chlorpyrifos on five vegetables in the controls were all lower

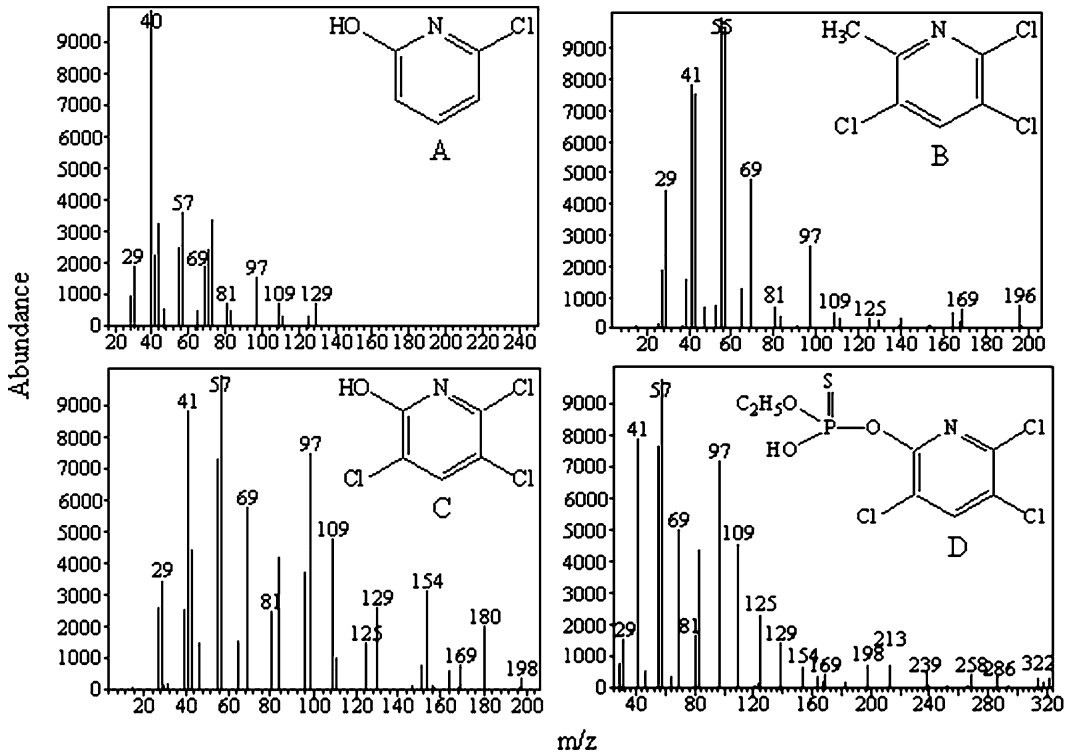

Figure 2. MS spectra and the proposed structures of four metabolites.

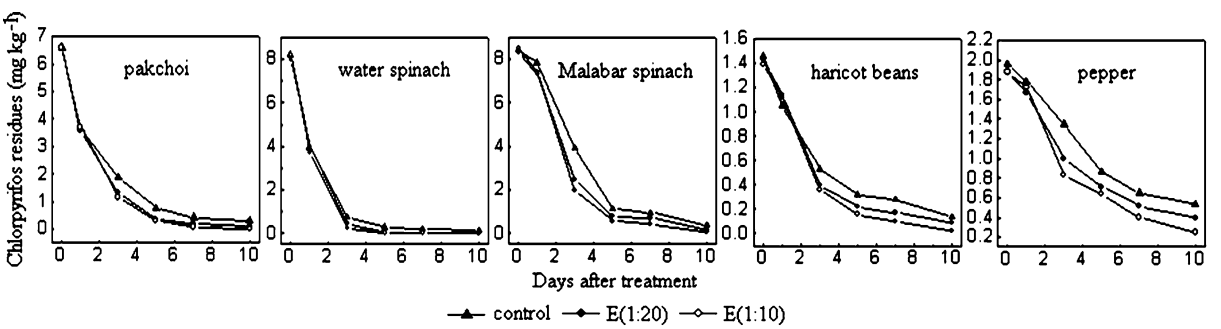

Figure 3. Degradation curves of chlorpyrifos by cell-free extracts on vegetables.

than the corresponding maximum residue levels (MRLs) of China (1.0 mg kg^{-1} on vegetables), but those on pakchoi, Malabar spinach, and pepper were higher than the corresponding MRLs of European Union (0.5 mg kg^{-1}, EU), and that on haricot beans also exceeded the corresponding EU MRLs (0.05 mg kg^{-1}). The R_7 value of chlorpyrifos on E (1:20) treated pakchoi was lower than the corresponding EU MRLs, and the R_7 values of chlorpyrifos on E (1:10) treated pakchoi, Malabar spinach, and pepper were lower than the corresponding EU MRLs.

The data indicated that cell-free extracts could rapidly degrade chlorpyrifos residues on vegetables. Furthermore, the degradation of chlorpyrifos was much faster in the treatments with E (1:10) than that in the treatments with E (1:20). After the treatments with E (1:10), the R_7 values of chlorpyrifos on five vegetables were all lower than the corresponding EU MRLs, except that the R_7 value (0.08 mg kg^{-1}) on haricot beans was slightly higher than the corresponding EU MRLs (0.05 mg kg^{-1}).

The results obtained suggested microbial cell-free extracts could efficiently remove or detoxify

Table 3. Statistic data of chlorpyrifos degradation on vegetables by cell-free extracts

Sample	Site	Disposal	Total degradation rate (mg (d kg vegetable)$^{-1}$)	DT_{50}[c] (days)	R_7 (mg kg^{-1})	r^2 [d]
Pakchoi	Open field	Control	0.630	2.19	0.55	0.9546
	Open field	E (1:20)[a]	0.647	1.54	0.23	0.9745
	Open field	E (1:10)[b]	0.656	1.18	0.10	0.9983
Water spinach	Open field	Control	0.808	1.69	0.26	0.8671
	Open field	E (1:20)	0.817	1.11	0.06	0.8927
	Open field	E (1:10)	0.821	0.73	0.01	0.9304
Malabar spinach	Greenhouse	Control	0.804	2.12	0.92	0.9649
	Greenhouse	E (1:20)	0.818	1.71	0.52	0.9744
	Greenhouse	E (1:10)	0.844	1.40	0.29	0.9807
Haricot beans	Greenhouse	Control	0.130	3.04	0.25	0.9673
	Greenhouse	E (1:20)	0.134	2.45	0.17	0.9530
	Greenhouse	E (1:10)	0.137	1.65	0.08	0.9895
Pepper	Greenhouse	Control	0.143	4.90	0.72	0.9711
	Greenhouse	E (1:20)	0.148	4.22	0.56	0.9742
	Greenhouse	E (1:10)	0.163	3.32	0.43	0.9805

[a]Cell-free extract with 1:20.
[b]Cell-free extract with 1:10.
[c]The degradation of chlorpyrifos on vegetables was described by the first-order function ($C = C_0 * e^{-k*t}$). The half-lives of degradation for chlorpyrifos (DT_{50}) on vegetables were obtained by the function $DT_{50} = \ln 2/k$.
[d]The degradation of chlorpyrifos on vegetables was simulated by the first-order function ($C = C_0 * e^{-k*t}$). The coefficients of determination r^2 were obtained by means of Microsoft Excel 2000.

chlorpyrifos residues on different vegetables. However, after application of microbial cell-free extracts, enzymatic activity was influenced by many factors, such as temperature, humidity, solar radiations, pH value and precipitation. The degradation rate of pesticide residues by microbial cell-free extracts on vegetables also depended largely on vegetable species, e.g., cell-free extracts were not readily adsorbed by haricot beans and pepper due to their smooth waxy surface and the morphology of the plant, thus the degradation rate of chlorpyrifos residues greatly decreased.

Conclusion

Degradation of chlorpyrifos by the fungal strain DSP of *Verticillium* sp. in mineral salt medium could be described with the first-order function. The cell-free extract from the fungal strain DSP in bran–glucose medium could enhance chlorpyrifos degradation on vegetables. The data of this study indicate that the cell-free extract from the fungal strain DSP is a promising approach for detoxification of chlorpyrifos on vegetables. However, further studies on environmental stability, production strategy, and safety of the cell-free extracts are required to establish efficient, safe and cost-effective large-scale process for destruction of chlorpyrifos residues on vegetables.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Nos. 20377036, 30230250, and 30270880), the Major State Basic Research Development Programme of China (2002CB410806), Zhejiang Provinicial Natural Science Foundation (301050) and Hangzhou Committee of Science and Technology (2002112A08).

References

Aguilera-del Real A, Valverde-García & Camacho-Ferre F (1999) Behavior of methamidophos residues in peppers, cucumbers, and cherry tomatoes grown in a greenhouse: evaluation by decline curves. J. Agric. Food Chem. 47: 3355–3358

Ahren D, Ursing BM & Tunlid A (1998) Phylogeny of nematode-trapping fungi based on 18S rDNA sequences. FEMS Microbiol. Lett. 158: 179–184

Barnett HL & Hunter BB (1972) Illustrated Genera of Imperfect Fungi. 3rd edn. Burgess Publishing Company, Minnesota, USA

Brown KA (1980) Phosphotriesterases of Flavobacterium sp. Soil Biol. Biochem. 12: 105–112

Bolognesi C & Morasso G (2000) Genotoxicity of pesticides: potential risk for consumers. Trends Food Sci. Technol. 11: 182–187

Chen W & Mulchandani A (1998) The use of live biocatalysts for pesticide detoxification. Trends Biotechnol. 16: 71–76

Cochran RC, Kishiyama J, Aldous C, Carr WC Jr & Pfeifer KF (1995) Chlorpyrifos: hazard assessment based on a review of the effects of short-term and long-term exposure in animals and humans. Food Chem. Toxicol. 33: 165–172

Dai FL (1987) Morphology and Taxonomy of Fungi. Science Press, Beijing, China

Dave KI, Miller CE & Wild JR (1993) Characterization of organophosphorus hydrolases and the genetic manipulation of the phosphotriesterase from *Pseudomonas diminuta*. Chem. Biol. Interact. 8: 55–68

Fan AM & Jackson RJ (1989) Pesticides and food safety. Regul. Toxicol. Pharm. 9: 158–174

Gajbhiye VT, Gupta S & Gupta RK (2004) Persistence of imidacloprid in/on cabbage and cauliflower. Bull. Environ. Contam. Toxicol. 72: 283–288

Garau VL, Angioni A, Real AAD, Russo M & Cabras P (2002) Disappearance of azoxystrobin, pyrimethanil, cyprodinil, and fludioxonil on tomatos in a greenhouse. J. Agric. Food Chem. 50: 1929–1932

Hernandez Torres ME, Egea Gonzalez FJ, Castro Cano ML, Moreno Frias M & Martinez Vidal JL (2002) Residues of methamidofos, malathion, and methiocarb in greenhouse crops. J. Agric. Food Chem. 50: 1172–1177

Horne I, Sutherland TD, Harcourt RI, Russell RJ & Oakeshott JG (2002) Identification of an opd (organophosphate degradation) gene in an *Agrobacterium* isolate. Appl. Environ. Microbiol. 68: 3371–3376

Lee SB & Taylor JW (1990) Isolation of DNA from fungal mycelia and single spores., In: Innis MA, Gelfand DH, Sninsky JJ & White TJ (Eds) PCR Protocols – A Guide to Methods and Applications, Academic Press, San Diego

Liu B, McConnell LL & Torrents A (2001) Hydrolysis of chlorpyrifos in natural waters of the Chesapeake Bay. Chemosphere 44: 1315–1323

Mallick K, Bharati K, Banerji A, Shakil NA & Sethunathan N (1999) Bacterial degradation of chlorpyrifos in pure cultures and in soil. Bull. Environ. Contam. Toxicol. 62: 48–54

Martinez Galera M, Gil Garcia MD, Rodriguez Lallena JA, Lopez T & Martinez Vidal JL (2003) Dissipation of pyrethroid residues in peppers, zucchinis, and green beans exposed to field treatments in greenhouses: evaluation by decline curves. J. Agric. Food Chem. 51: 5745–5751

Martínez Vidal JL, Egea González FJ, Martínez Galera M & Castro Cano ML (1998) Diminution of chlorpyrifos and chlorpyrifos oxon in tomatoes and green beans grown in greenhouses. J. Agric. Food Chem. 46: 1440–1444

Oliver GR, Bolles HG & Shurdut BA (2000) Chlorpyrifos: probabilistic assessment of exposure and risk. Neurotoxicology 21: 203–208

Racke KD, Coats JR & Titus KR (1988) Degradation of chlorpyrifos and its hydrolysis product, 3,5,6-trichloro-2-pyridinol in soil. J. Environ. Sci. Health 23: 527–539

Robertson LN, Chandler KJ, Stickley BDA, Cocco RF & Ahmetagic M (1998) Enhanced microbial degradation implicated in rapid loss of chlorpyrifos from the controlled release formulation suSucon (R) Blue in soil. Crop Prot. 17: 29–33

Serrano R, Lopez FJ, Hernandez F & Pena JB (1997) Bioconcentration of chlorpyrifos, chlorfenvinphos and methidathion in Mytilus galloprovincialis. Bull. Environ. Contam. Toxicol. 59: 968–975

Singh BK, Walker A, Morgan AW & Wright DJ (2003) Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl. Environ. Microbiol. 69: 5198–5206

Singh BK, Walker A, Morgan JA & Wright DJ (2004) Biodegradation of chlorpyrifos by enterobacter strain B-14 and its use in bioremediation of contaminated soils. Appl. Environ. Microbiol. 70: 4855–4863

Tweedy BG, Dishburger HJ, Ballantine LG, McCarthy J & Murphy J (1991) Pesticide Residues and Food Safety: A Harvest of Viewpoints. American Chemical Society, Washington, DC

van Lenteren JC (2000) A greenhouse without pesticides: fact or fantasy?. Crop Prot. 19: 375–384

Velasco-Arjona A, Manclus JJ, Montoya A & de Luque Castro MD (1997) Robotic sample pretreatment-immunoassay determination of chlorpyrifos metabolite (TCP) in soil and fruit. Talanta 45: 371–377

White TJ, Bruns T & Lee S (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics., In: Innis MA, Gelfand DH, Sninsky JJ & White TJ (Eds) PCR Protocols – A Guide to Methods and Applications,, Academic Press, San Diego

Zayed SMAD, Farghaly M & El-Maghraby S (2003) Fate of ^{14}C-chlorpyrifos in stored soybeans and its toxicological potential to mice. Food Chem. Toxicol. 41: 767–772