Biodegradation (2006) 17: 275–283
DOI 10.1007/s10532-005-7130-2

Isolation of fenitrothion-degrading strain *Burkholderia* sp. FDS-1 and cloning of *mpd* gene

Zhonghui Zhang‡, Qing Hong‡, Jianghong Xu, Xiaozhou Zhang & Shunpeng Li*
*Key Lab of Microbiological Engineering of Agricultural Environment, Ministry of Agriculture, College of Life Science, Nanjing Agricultural University, 210095, Nanjing, Jiangsu Province, P.R. China (*author for correspondence: e-mail: lsp@njau.edu.cn)*

Accepted 11 May 2005

Key words: biodegradation, *Burkholderia*, fenitrothion, *mpd* gene

Abstract

A short rod shaped, gram-negative bacterium strain *Burkholderia* sp. FDS-1 was isolated from the sludge of the wastewater treating system of an organophosphorus pesticides manufacturer. The isolate was capable of using fenitrothion as the sole carbon source for its growth. FDS-1 first hydrolyzed fenitrothion to 3-methyl-4-nitrophenol, which was further metabolized to nitrite and methylhydroquinone. The addition of other carbon source and omitting phosphorus source had little effect on the hydrolysis of fenitrothion. The gene encoding the organophosphorus hydrolytic enzyme was cloned and sequenced. The sequence was similar to *mpd*, a gene previously shown to encode a parathion-methyl-hydrolyzing enzyme in *Plesiomonas* sp. M6. The inoculation of strain FDS-1 (10^6 cells g^{-1}) to soil treated with 100 mg fenitrothion emulsion kg^{-1} resulted in a higher degradation rate than in noninoculated soils regardless of the soil sterilized or nonsterilized. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Introduction

Organophosphorus pesticides such as parathion, parathion-methyl and fenitrothion are a group of highly toxic agricultural chemicals and widely used to control a wide range of insect pests. These insecticides inhibit acetyl cholinesterase in an irreversible manner and cause insect death. As the acetyl cholinesterase is present in all vertebrates, the potential for damage by this class of insecticides to nontarget organisms is very high. Bioremediation is considered to be a major method of degrading contaminants in the environment and can take various forms. Investigations of microbial degradation are useful in the development of strategies for the detoxification of the insecticides by microorganisms. Many researchers have isolated strains that can hydrolyze organophosphorus pesticides. Two parathion-degrading bacteria *Pseudomonas diminuta* GM and *Flavobacterium* sp. ATCC27551 have been isolated in Philippines and USA (Serdar et al. 1982; Mulbry et al. 1986). *Agrobacterium radiobacter* P230 was isolated in Australia, which could hydrolyze a wide range of orgranophosphorus pesticides (Horne et al. 2002). A parathion-methyl-degrading bacterium M6 was isolated in P.R. China (Cui et al. 2001). A fenitrothion-degrading bacterium *Burkholderia* sp. NF100 was isolated in Japan (Masahito et al. 2000). These bacteria all possess organophosphorus pesticide hydrolase (OPH) activity. The OPH encoding genes *opd* in *Pseudomonas diminuta* GM, *Flavobacterium* sp. ATCC27551, *Agrobacterium radiobacter* P230 and *mpd* in *Plesiomonas* sp. M6 were cloned and sequenced (Serdar et al. 1982; Mulbry et al. 1986; Cui et al. 2001; Horne et al. 2002; Siddavattam et al. 2003). However, this is the first report of cloning *mpd* gene from the genus

‡ Both authors contributed equally to this work.

Burkholderia and investigating the degradation of fenitrothion in soils by bacterial inoculation.

Materials and methods

Chemicals and pesticide analysis

Analytical-grade fenitrothion (*O,O*-dimethyl *O*-*p*-nitro-*m*-tolyl phosphorothioate), parathion (*O,O*-diethyl *O*-4-nitrophenyl phosphorothioate), parathion-methyl (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) (99.0% purity; Beijing Kang-Lin Science & Technology Co. Ltd., Beijing, P.R. China), 3-methyl-4-nitrophenol (MNP) (99% purity; Shanxi JinYang Chem Co. Ltd., Shanxi, P.R. China) and methylhydroquinone (MHQ) (99.5% purity; Jiangsu JinHuang Tech & Chem Co. Ltd., Jiangsu, P.R. China) were used throughout this study. For the solubility of fenitrothion was very low (fenitrothion: 14 mg l^{-1} at room temperature), 10 g pesticides was added into 30 ml Tween 80, ultrasonicated to confect fenitrothion emulsion, stored at room temperature. All samples were analyzed as follows: a 5 ml aliquot of the sample was extracted with 10 ml dichloromethane. The extracts were dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure using a centrifugal evaporator at room temperature. The residual organic material was redissolved in 5 ml of acetonitrile, and 20 μl of the resulting solution was subjected to reverse-phase high-performance liquid chromatography (HPLC, 600 Controller, Rheodyne 7725i Manual injector and 2487 Dual λ Absorbance Detector; Waters Co., Milford, MA). The separation column for the HPLC (internal diameter, 4.6 mm; length, 25 cm) was filled with Kromasil 100-5C18. The mobile phase was acetonitrile:water (70:30, v:v), and the flow rate was 1.0 ml min^{-1}. Detections of fenitrothion, parathion, parathion-methyl, MNP and MHQ were recorded at 270 nm and their retention times were 6.265, 5.482, 8.165, 3.357 and 2.498, respectively. Nitrite was determined by the modified Griess–Ilosvay method (Montgomery & Dymock 1961).

Isolation of a fenitrothion-degrading bacterium by enrichment culture

To isolate bacteria capable of degrading fenitrothion, a soil enrichment technique was used. About 1.0 g of sludge was added to an Erlenmeyer flask (250 ml) containing 100 ml mineral salt medium (MSM, which contained NaCl 1.00 g l^{-1}, NH_4NO_3 1.00 g l^{-1}, K_2HPO_4 1.50 g l^{-1}, KH_2PO_4 0.50 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.10 g l^{-1}, $FeSO_4$ 0.025 g l^{-1}, pH 7.0) with the addition of fenitrothion emulsion to a final concentration of 100 mg l^{-1} as carbon source and incubated at 30 °C on a rotary shaker at 200 rpm for 7 days. About 1 ml of the suspension was then transferred to fresh MSM containing 100 mg fenitrothion emulsion l^{-1} and incubated for 7 days. After three rounds of enrichment, the culture was diluted with sterilized 0.1 M phosphate buffer (pH 7.5) by the method of a series of dilution. The 10^{-5}–10^{-8} diluted suspensions were plated on MSM plates containing 100 mg fenitrothion emulsion l^{-1} and 1.5% agar. After 3 days incubation at 30 °C, microbial colonies became visible, and a clear halo appeared around colonies capable of degrading fenitrothion. We selected and purified these colonies and tested their degrading capability by inoculation in liquid medium (100 ml in a 250 ml-Erlenmeyer flask). The incubation conditions were the same as described above.

Characterization of isolate FDS-1

The isolate was tested for the oxidase production, nitrate reduction, hydrolysis of starch and hydrolysis of gelatin (Xiuzhu & Miaoying 2001). Gram strain, shape, and motility under the microscope were also examined (Xiuzhu & Miaoying 2001). The guanine plus cytosine (G + C) content of bacterial DNA was determined as described by Tamaoka and Komagata (1984). The 16S rDNA gene of this isolate was cloned and analyzed as the following procedure. Cells were grown overnight on Luria-Bertani medium (LB) (Sambrook et al. 1989). The cells were harvested during the later exponential phase by centrifugation at $6000 \times g$ for 5 min and washed twice with sterilized 0.1 M TE buffer (pH 8.0), then the genomic DNA was extracted by the method of high-salt-concentration precipitation (Miller et al. 1988). The 16S rDNA gene of the isolate was amplified with a set of universal primers (BioAsia, Shanghai, P.R. China) that allows amplification of most bacterial 16S rDNA genes. The primers 8f (5′-CACGGATC-CAGACTTTGATYMTGGCTCAG-3′, forward) and 1512r (5′-GTGAAGCTTACGGYTAGCTT-GTTACGACTT-3′, reverse) were used. After

amplification, 5 μl of each reaction mixture was run on 0.7% (w/v) agarose gel to confirm the size and purity of PCR products. The DNA was then purified with a DNA Gel Extraction Kit following the protocol provided by the supplier (V-gene, Biotechnology Ltd., Hangzhou, P.R. China). The purified DNA fragment was ligated into T-Vector (TAKARA, BIOT Ltd., Dalian, P.R. China) and transformed into *E. coli* DH5α cells. The recombinant plasmid in positive clones was extracted and used as the template for direct sequence of 16S rDNA fragment in TARAKA Biot Ltd. The DNA was analyzed for similarity to other 16S rDNA sequences, using the BLAST program of the National Center for Biotechnology Information (NCBI) database (Altschul et al. 1990). Selected sequences with the greatest sequence similarity to the isolate sequence were extracted from the database and aligned. The software Bioedit did sequence analysis and the tree was generated by Drawtree (all from within the PHYLIP and RDP suite of packages).

Inoculum preparation for degradation studies

Unless otherwise stated, the inoculum for all of the experiments was prepared by growing bacteria in 50 ml of LB medium overnight at 30 °C at 200 rpm on a rotary shaker. Cells were harvested by centrifugation at 6000 × *g* for 5 min. Cells were washed twice with 25 ml of sterilized 0.5 M phosphate buffer (pH 7.5) and quantified by the dilution plate count technique. For all experiments, 10^6 cells ml^{-1} were used and samples were incubated at 30 °C at 200 rpm unless otherwise stated.

Degradation of fenitrothion by isolate FDS-1 in cell culture

To determine the degradation pathway of fenitrothion, strain FDS-1 was inoculated into MSM with the addition of fenitrothion emulsion to a final concentration of 100 mg l^{-1} and incubated at 30°C at 200 rpm in a rotary shaker. Samples of culture medium were periodically withdrawn and pesticide residue measurement was done as described above. Effects of nutrient composition, incubation temperature (10, 20, 25, 30, 35, and 40 °C), initial pesticide concentration (10, 50, 100, 200, and 500 mg l^{-1}), inoculum density (10^2, 10^3, 10^4, 10^5, 10^6, and 10^7cells ml^{-1}), and medium pH (4.0, 5.0, 6.0, 7.0, and 8.0) were studied. To examine the effect of nutrient composition on the fenitrothion-degrading ability of the isolated bacterium, the MSM medium with fenitrothion (100 mg l^{-1}) was supplemented with extra carbon sources. Active degrading culture (1 ml) was transferred to 100 ml of the following media: MSM containing 100 mg fenitrothion emulsion l^{-1}, MSM containing 100 mg fenitrothion emulsion l^{-1} and glucose. Glucose was added as 0.5-ml filter-sterile solutions in distilled water to a final concentration of 1 g l^{-1}. About 20 mg l^{-1} parathion and 40 mg l^{-1} parathion-methyl was added to MSM to investigate their degradation. Triplicate of each composition without inoculation were used as controls.

Degradation of fenitrothion by isolate FDS-1 in soil

A soil sample from the vegetable field at Nanjing, P.R. China was used in this study. This soil had characteristics of a sandy loam with 67% sand, 12% silt and 15% clay, 8.27% organic matter, and a pH of 5.6. Soil samples (1 kg) were sterilized at 121 °C for 30 min. Similar soil samples were stored at 4 °C. Both sterilized and nonsterilized soil (100 g) were treated under aseptic conditions with 100 mg fenitrothion emulsion kg^{-1} and divided into two sets. One set of sterilized and nonsterilized soils in triplicate was inoculated with fenitrothion-degrading bacterium (10^6 cells g^{-1}), and the second set without inoculation was kept as controls. The inoculum was thoroughly mixed into soils under sterile conditions. Soil samples were incubated at 30 °C and 50% water-holding capacity in the dark.

The fenitrothion concentration in the soil samples was analyzed at different time points between 0 and 9 days. The soil sample containing fenitrothion was analyzed using the method as follows: the soil sample (5 g) and 5 g anhydrous Na_2SO_4 were placed in 20 ml glass vial. Dichloromethane (10 ml) was added before sealing the vial with a Teflon-lined screw cap. The sealed vial was placed on the vortex for 30 s and then in a sonicating bath for 10 min before remixing on the vortex remixer for about 15 s. It was then placed in the sonicating bath for another 10 min. The solvent was transferred to a clean, dry vial containing 1 g activated FlorosilTM (Sigma) and

0.6 ml water (i.e. 6% water (w/w)). The sealed vial was shaken for 1 min and allowed to stand overnight at ambient temperature. This silica "clean-up" procedure was used to remove interfering humic materials. The extracts was finally filtered through a 0.45 μm fiber filter. The extract were dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure using a centrifugal evaporator at room temperature. The residual organic material was redissolved in 5 ml of acetonitrile, and 20 μl of the resulting solution was subjected to HPLC for detection.

Cloning and expression the mpd gene from isolate FDS−1

Plasmids pUC18 and pET29a (Novagen, Madison, WI, USA) were used as cloning and expression vectors, respectively. *Escherichia coli* DH5α and BL21 (DE3) (Novagen) were used as recombinant hosts of the cloning and expressing plasmids, respectively. Genomic DNA of isolate FDS-1was extracted by the method as described above and digested partially with *Sau*3AI. The 1- to 5-kb fragments were recovered by DNA Gel Extraction Kit (V-gene Biotechnology Ltd., Hangzhou, P.R. China) and ligated into *Bam*HI-digested pUC18. The ligation product named pTX was transformed into *E. coli* DH5α cells. The cells were then plated on LB containing 100 mg l^{-1} ampicillin and 150 mg fenitrothion emulsion l^{-1} and incubated at 37 °C for 16 h. The positive clones that produced clear yellow halos were selected.

The entire *mpd* structural gene of isolate FDS-1 was amplified by PCR with a pair of primers designed according to the deduced sequence of *mpd* gene. The PCR primers were mpdfp (5′-GAATTCATATGGCAGCACCGCAGGTG-CGCACCTCG-3′, forward) and mpdrp (5′-GAATTCTCGAGCTTGGGGTTGACGACCG-3′, reverse). The *Nde*I and *Xho*I sites (underlined) were incorporated into primers to facilitate directional cloning of the PCR product into (*Nde*I–*Xho*I sites of the expression vector) pET29a (Novagen). The resulting plasmid was designated as pE1 and transformed into *E. coli* BL21 (DE3). Overnight culture of *E. coli* strain BL21 (DE3) (pE1) was subcultured into 50 ml of LB medium and allowed to grow until the culture density, measured by the absorbance at 600 nm, reached 0.5. The *mpd* gene expression was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM, and 1, 2, 3, 4 h later protein extracts prepared from these cultures were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.0% SDS-PAGE).

The detection of plasmid DNA

The plasmid DNA in the isolate FDS-1 was detected with the methods as described in detail previously (Kado & Liu 1981; Sambrook et al. 1989).

Results

Isolation and characterization of fenitrothion-degrading bacterium FDS-1

Bacterial isolates with the ability of degrading fenitrothion were obtained from the enrichment. The isolate FDS-1 was selected out of five isolates for its relatively high growth and degrading efficiency. Taxonomic properties of FDS-1 were as follows: a rod-shaped bacterium with dimensions of 1.0–1.6 μm in length and 0.3–0.6 μm in width without flagellum; gram-negative; oxidase production, positive; nitrate reduction, positive; hydrolysis of starch, negative; hydrolysis of gelatin, positive; G + C content, 66.5 ± 2.5%. The sequence of 1497 bp fragment of the 16S rDNA gene of FDS-1 was deposited at the GenBank under Accession No. AY550913. After comparing the sequence with those in the Genbank database by an online alignment search, a dendrogram illustrating the results of the 16S rDNA analysis using PHYLIP is presented in Figure 1. The result showed that this sequence was 99% identical to that of the 16S rDNA gene of *Burkholderia* sp. AK-5 (Genbank Accession No. AB103080), 99% similar to *Burkholderia* sp. strain JRB1 (GenBank Accession No. AF439776), 98% similar to *Burkholderia* sp. strain S4.9 (GenBank Accession No. AF247496) and 97% similar to *Burkholderia* sp. strain NF100 (GenBank Accession No. AB025790). Based on these characters, the isolate FDS-1 was preliminary identified as a *Burkholderia* sp.

Fenitrothion degradation ability of strain FDS-1

The kinetics of degradation of fenitrothion and its metabolites and growth of FDS-1 were monitored

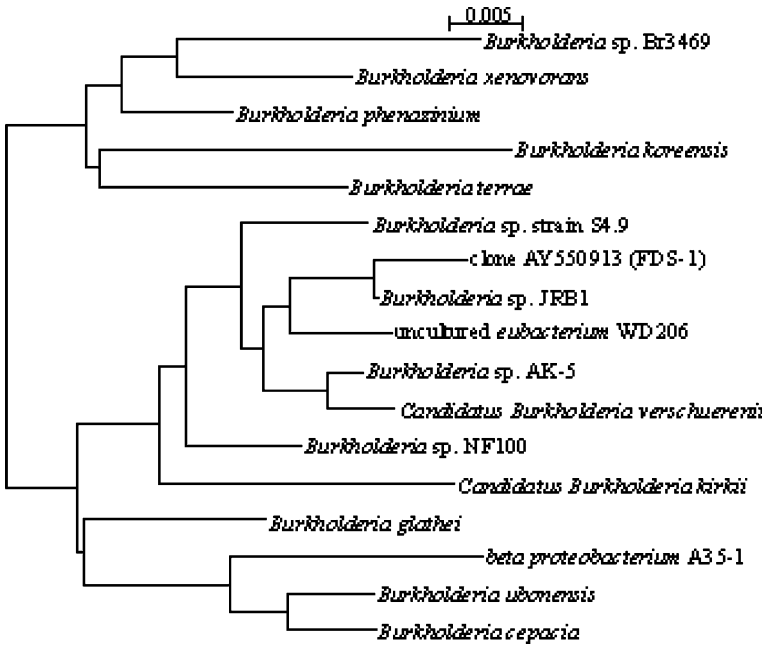

Figure 1. Dendrogram illustrating the (16S rDNA gene) similarity of the fenitrothion-degrading bacterium (FDS-1) to members of the genus *Burkholderia* and (its closest) relatives.

for 14 h after inoculation of FDS-1 (Figure 2). Fenitrothion was completely hydrolyzed to MNP by FDS-1 within 1 h, but the growth of FDS-1was not detected. The concentration of MNP increased and then disappeared completely at between 1 and 12 h of growth, and MHQ was detected in the culture during the logarithmic growth phase. Nitrite production continued from 2 to 13 h and ceased at 14 h. Degradation of fenitrothion was negligible in MSM medium containing 100 mg fenitrothion emulsion l^{-1} without the addition of FDS-1 strain. The isolate was not capable of utilizing the emulsion Tween 80 as a carbon source in MSM medium (data not shown). These

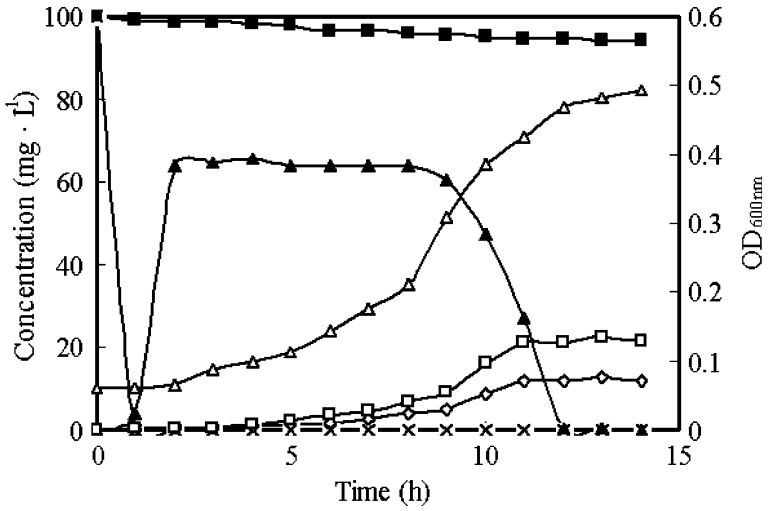

Figure 2. Utilization of fenitrothion as a sole source of carbon for growth by *Burkholderia* sp. FDS-1. ■, Control; Δ, $OD_{600\ nm}$; ×, Fenitrothion; ▲, MNP; □, MHQ; ◇, Nitrite. The standard error was within 5% of the mean.

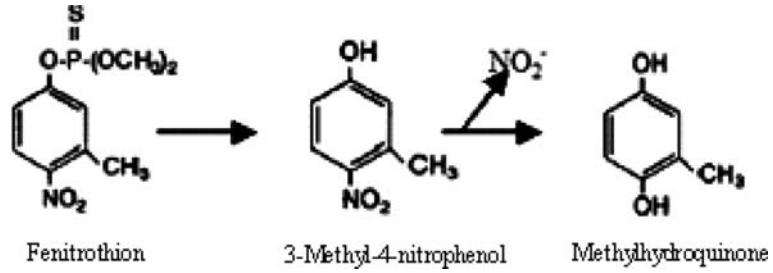

Figure 3. Proposed pathway of fenitrothion degradation by *Burkholderia* sp. FDS-1.

observations indicated that the metabolism of fenitrothion in strain FDS-1 was initiated by its hydrolysis to MNP, which was metabolized to nitrite and MHQ (Figure 3).

Fenitrothion was hydrolyzed completely within 1 h in MSM and MSM plus glucose media. The hydrolysis patterns of fenitrothion were not affected by the presence of a carbon supplement. Omitting a phosphorus source had no obvious effect on the hydrolysis of fenitrothion (data not shown). The most rapid degradation of fenitrothion and MNP was observed at 30 °C. The degradation rates were similar at 25 and 35 °C. The rate dropped significantly when the temperature was further decreased to 10 °C or increased to 40 °C (data not shown). The initial pesticide concentration had little effect on the hydrolysis of fenitrothion, but when the concentration was 200 mg l^{-1} or above there was no degradation of MNP and no growth of the isolate. At the high inoculum density ($>10^5$ cells ml^{-1}), fenitrothion was hydrolyzed completely within 1 h and MNP was degraded within 12 h. Degradation was slow initially at lower cell density. However, after initial lag phase, rapid degradation occurs. The slight hydrolysis of fenitrothion and no degradation of MNP were observed when the isolate was inoculated at a very low density (10^2 and 10^3 cells ml^{-1}). Degradation of fenitrothion was elevated with an increase in medium pH, although the differences were little, except at pH 4.0, where degradation was very slow. Degradation of fenitrothion was negligible in all controls irrespective of medium pH (data not shown). Other organophosphorus insecticides parathion and parathion-methyl were hydrolyzed by the isolate FDS-1, as evidenced by the accumulation of the hydrolysis products (data not shown).

Degradation of fenitrothion and MNP in soil by FDS-1

The inoculation of FDS-1 to vegetable field soils resulted in a rapid rate of fenitrothion degradation. Degradation of fenitrothion in control nonsterilized soils (without inoculation) was minimal where less than 25% of the applied fenitrothion was degraded over a 9-day period incubation. Degradation of fenitrothion was insignificant in control-sterilized soils. The primary metabolite MNP was observed to accumulate in soils. MNP was stable whether sterilized or nonsterilized in soils (data not shown). However, the inoculation of FDS-1 to soils showed a more rapid rate of MNP degradation (Figure 4).

Cloning and nucleotide sequence analysis of the mpd gene

Two positive clones that produced clear yellow halos were selected. The recombinant plasmids harbored by them were designated as pT2 and pT3. They carried a 4.8- and 1.35-kb inserts, respectively, and restriction map analysis indicated that the 1.35-kb fragment was included in the 4.8-kb fragment (data not shown). The insert of pT3 was sequenced in both directions and analyzed by software Bioedit. Its exact length was 1324 bp. The molecular size of the *mpd* gene product of *Plesiomonas* sp. M6 was about 35 kDa (Cui et al. 2001), so we deduced the region ranging from 135 to 1109 as the structural gene sequence of *mpd* gene. Expression of the *mpd* gene in BL21 (DE3) (pE1) revealed the synthesis of a protein of approximately 35 kDa (Figure 5). The crude enzyme of BL21 (DE3) (pE1) extracted by the method of ultrasonic crush was dipped on the

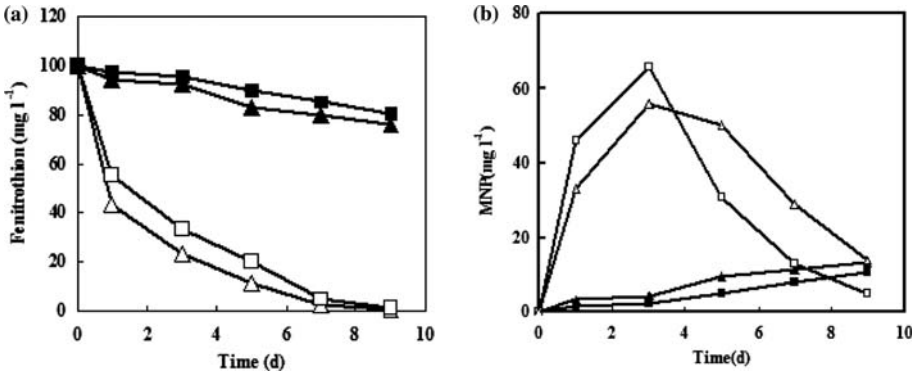

Figure 4. Degradation of fenitrothion (a) and accumulation of MNP (b) in sterilized and nonsterilized soils inoculated with fenitrothion-degrading strain FDS-1 at the rate of 10^6 cells g^{-1}. ■, control in sterilized soils; □, sterilized soil; ▲, control in nonsterilized soils; Δ, nonsterilized soil. The standard error was within 5% of the mean.

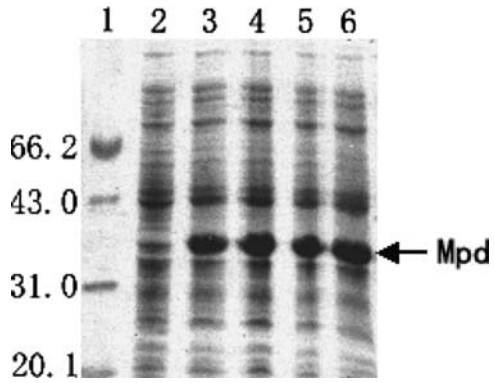

Figure 5. Expression of *mpd* gene in *E. coli*. Lane 1, molecular mass markers (in kilodaltons); lane 2, protein extracts prepared from uninduced cultures of *E. coli* BL21 (DE3) (pE1) cells; lanes 3–6, protein extracts prepared from induced cultures of *E. coli* BL21 (DE3) (pE1) cells for 1, 2, 3 and 4 h, respectively. Expression of *mpd* is indicated by arrow.

MSM plate with addition of fenitrothion emulsion to a final concentration to 100 mg l^{-1}. A clear yellow halo was shown and the result indicated successful expression of the *mpd* gene in *E. coli*. The control extract of *E. coli* without the expression vector had no hydrolysis halo. It suggested that the sequence region ranging from 135 to 1109 was the true structural sequence of *mpd* gene. This sequence had been deposited at the GenBank under Accession No. AY646835. The gene was 99% similar to *mpd* gene (GenBank Accession No. AF338729) of *Plesimonas* sp. M6 (Cui et al. 2001), 99% similar to *mpd* gene of *Pseudomonas* sp. WBC-3 (GenBank Accession No. AY251554) and 99% similar to *mpd* gene of *Ochrobactrum* sp. mp-6 (GenBank Accession No. AY627038) on the nucleic acid level.

The detection of plasmid DNA

No plasmid was detected in strain FDS-1 by the method as described above, which indicated that the *mpd* gene was chromosome based.

Discussion

In the present study, the strain FDS-1 was isolated from contaminated sludge. The isolate showed the greatest similarity to members of the genus *Burkholderia*. So far only one strain NF100 of *Burkholderia* has been reported to degrade organophosphorus pesticides (Masahito et al. 2000). Previous reports have suggested that bacteria mainly degrade these compounds cometabolically (Mulbry et al. 1986; Richnis et al. 1997; Cui et al. 2001; Horne et al. 2002). Some species of bacteria have been isolated that can utilize organophosphates as a source of carbon or phosphorus (Rosenberg & Alexander 1979). The bacterium isolated in this study has very strong organophosphate hydrolase (OPH) activity and hydrolyzed 100 mg fenitrothion emulsion l^{-1} within 1 h when inoculated with 10^6 cells ml^{-1}. Addition of other carbon and phosphorus source has no obvious effect on the hydrolysis of fenitrothion.

Fenitrothion and MNP degradation by the isolate FDS-1 were very rapid at temperatures ranging from 20 to 30 °C. The peak of degradation rate was observed at 30 °C and the slowest was observed at 10 and 40 °C. These results were expected, since

most members of the genus *Burkholderia* have the highest growth rate at temperatures with a range from 25 to 30°C. The concentration of pesticide has no apparent effect on the hydrolysis of fenitrothion within 1 h, even at a level as high as 500 mg l^{-1}. Degradation of different pesticides at high concentration by isolated microorganisms has been reported earlier (Struthers et al. 1998; Karpouzas & Walker 2000). The result indicated that the *Burkholderia* strain isolated in this study could be tolerant to high concentration of fenitrothion. However, when the pesticide concentration was higher than 200 mg l^{-1}, the strain stopped degrading MNP and no growth of the isolate was detected. It was at least in part due to that MNP had antimicrobial activity at high concentrations (data not shown).

The molecular basis of degradation of certain organophosphates has been studied extensively (Serdar et al. 1982; Mulbry et al. 1986; Horne et al. 2002; Siddavattam et al. 2003). A widely distributed organophosphate-degrading gene *opd* was isolated from temporally, geographically and biologically different species (Sethunathan & Yoshida 1973; Serdar et al. 1982; Horne et al. 2002). Most of the *opd* genes are plasmid-based (Mulbry et al. 1986; Harper et al. 1988). Two *opd* genes from *P. diminuta* and *Flavobacterium* sp. strain ATCC27551 have been cloned and sequenced. Recently, Horne et al. isolated a chromosome-based *opd* gene from *A. radiobacter*, which shows DNA sequence homology with the *opd* genes described above (Horne et al. 2002). In the isolate FDS-1, no plasmid DNA was detected. This suggests that the *mpd* gene may be chromosome based. Masahito et al. (2000) isolated *Burkholderia* sp. strain NF100, which is capable of utilizing fenitrothion as a sole source of carbon, and characterized that the fenitrothion-degrading capability of the isolate was associated with the two plasmids harbored by this bacterium. However, the *mpd* gene was not cloned (Masahito et al. 2000). Therefore this is the first report of cloning the *mpd* gene from the genus of *Burkholderia*.

Bioremediation is a cost effective method to degrade toxic compounds into innocuous products. Successful removal of pesticides by inoculation of bacteria (bioaugmentation) had been reported earlier for many compounds (Barles et al. 1979; Kearney et al. 1986; Mulbry et al. 1996; Struthers et al. 1998; Karpouzas & Walker 2000) In this study, the addition of strain FDS-1 (10^6 cells g^{-1}) to soil with a low indigenous population of fenitrothion-degrading bacteria treated with 100 mg fenitrothion emulsion kg^{-1} also resulted in a higher degradation rate than was observed in soils without inoculation. OPH is believed to be an ideal enzyme for bioremediation of organophosphorus pesticides because of its ability to hydrolyze the compounds at a rate approaching the diffusion limits (Scanlan & Reid 1995). The *mpd* gene of strain FDS-1 was cloned and expressed, which may supply OPH products at low cost for environment products. These results highlight the potential of this bacterium to be used in the cleanup of contaminated pesticide waste in the environment.

Acknowledgements

This work was supported by Chinese National Programs for High Technology Research and Development (2003AA241150), Chinese National Natural Science Fund (30300005, 30400013, and 40471073), the Project for National Programs for Science and Technology Development of Jiangsu Province (BE2002345, BE2003343).

References

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. J. Mol. Biol. 215: 403–410

Barles RM, Topp EE & Blackwell BA (1979) Accelerated parathion degradation in soil inoculated with acclimated bacteria under field conditions. Arch. Environ. Contamin. Toxicol. 8: 647–660

Cui ZL, Li SP & Fu G (2001) Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. Appl. Environ. Microbiol. 67: 4922–4925

Harper LL, McDaniel CS, Miller CE & Wild JR (1988) Dissimilar plasmids isolated from *Pseudomonas diminuta* GM and a *Flavobacterium* sp. (ATCC 27551) contain identical *opd* genes. Appl. Environ. Microbiol. 54: 2586–2589

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

Kado CI & Liu ST (1981) Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. 145: 1365–1373

Karpouzas DG & Walker A (2000) Factors influencing the ability of *Pseudomonas putida* epI to degrade ethoprophos in soil. Soil Biol. Biochem. 32: 1753–1762

Kearney PC, Karns JS, Muldoon MT & Ruth JM (1986) Coumaphos disposal by combined microbial and UV-ozonation reactions. J. Agric. Food Chem. 34: 702–706

Masahito H, Motoko H & Shinichi T (2000) Involvement of two plasmids in fenitrothion degradation by *Burkholderia* sp. Strain NF100. Appl. Environ. Microbiol. 66: 1737–1740

Miller SA, Dykes DD & Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16: 1215

Montgomery HAC & Dymock JF (1961) The determination of nitrite in water. Analysis 86: 414–416

Mulbry WW, Karns JS, Kearney PC, Nelson JO, McDaniel CS & Wild JR (1986) Identification of a plasmid-borne parathion hydrolase gene from *Flavobacterium* sp. by Southern hybridization with *opd* from *Pseudomonas diminuta*. Appl. Environ. Microbiol. 51: 926–930

Mulbry WW, Valle PLDel & Karns JS (1996) Biodegradation of the organophosphate insecticide coumaphos in highly contaminated soils and in liquid waters. Pestic. Sci. 48: 149–155

Richnis R, Kaneva A, Mulchandani A & Chen W (1997) Biodegradation of organophosphorus pesticides using surface-expressed organophosphorus hydrolase. Nat. Biotechol. 15: 984–987

Rosenberg A & Alexander M (1979) Microbial cleavage of various organophosphorus insecticides. Appl. Environ. Microbiol. 37: 886–891

Sambrook J, Fritsch EF & Maniatis T (1989) Molecular Cloning: A Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York 908–910

Scanlan CS & Reid RC (1995) Evolution in action. Chem. Biol. 2: 71–75

Serdar CM, Gibson DT, Munnecke DM & Lancaster JH (1982) Plasmid involvement in parathion hydrolysis by *Pseudomonas diminuta*. Appl. Environ. Microbiol. 44: 246–249

Sethunathan NN & Yoshida T (1973) A *Flavobacterium* that degrades diazinon and parathion. Can. J. Microbiol. 19: 873–875

Siddavattam D, Khajamohiddin S, Manavathi B, Pakala SB & Merrick M (2003) Transposon-like organization of the plasmid-borne organophosphate degradation (*opd*) gene cluster found in *Flavobacterium* sp. Appl. Environ. Microbiol. 69: 2533–2539

Struthers JK, Jayachandran K & Moorman TB (1998) Biodegradation of atrazine by *Agrobacterium radiobacter* J14a and use of this strain in bioremediation of contaminated soil. Appl. Environ. Microbiol. 64: 3368–3375

Tamaoka J & Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol. Lett. 25: 125–128

Xiuzhu D & Miaoying C (2001) Manual of Systematical and Determinative Bacteriology. Science press, Beijing 370–390