Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 19(2007) 1257–1260
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Biodegradation of methyl parathion by *Acinetobacter radioresistens* USTB-04

LIU Fang-yao, HONG Ming-zhang, LIU Dan-mei, LI Ya-wen, SHOU Pei-shun, YAN Hai*, SHI Guo-qing

Department of Biological Science and Technology, School of Applied Science, University of Science and Technology Beijing, Beijing 100083, China. E-mail: haiyan@sas.ustb.edu.cn

Received 9 October 2006; revised 16 January 2007; accepted 23 April 2007

Abstract

Biodegradation of methyl parathion (MP), a widely used organophosphorus pesticide, was investigated using a newly isolated bacterium strain *Acinetobacter radioresistens* USTB-04. MP at an initial concentration of 1200 mg/L could be totally biodegraded by *A. radioresistens* USTB-04 as the sole carbon source less than 4 d in the presence of phosphate and urea as phosphorus and nitrogen sources, respectively. Biodegradation of MP was also achieved using cell-free extract of *A. radioresistens* USTB-04. MP at an initial concentration of 130 mg/L was completely biodegraded in 2 h in the presence of cell-free extract with a protein concentration of 148.0 mg/L, which was increased with the increase of pH from 5.0 to 8.0. Contrary to published reports, no intermediate or final degradation metabolites of MP could be observed. Thus we suggest that the cleavage of C–C bond on the benzene ring other than P–O bond may be the biodegradation pathway of MP by *A. radioresistens* USTB-04.

Key words: *Acinetobacter radioresistens* USTB-04; biodegradation; methyl parathion

Introduction

Many kinds of pesticides including methyl parathion (MP) are increasingly produced and released to the environment at a growing rate, which may pollute the soil, water and air in the world if it is not used properly. MP is a widely used organophosphorus pesticide, which is frequently detected in both terrestrial and aquatic environments (Tsatsakis *et al*., 2002). Because of its potent toxicity to both wildlife and humans, and recalcitrant to be biodegraded in naturally indigenous microorganisms, MP was classified as both a persistent organic pollutant and environmental endocrine-disrupting chemical (Ang *et al*., 2005). The neuro-toxicological property of MP was found to suppress the activity of acetyl-cholinesterase, and as a result, prevent acetyl-cholinesterase from breaking down acetylcholine at the synaptic junction (Ohshiro *et al*., 1999). MP can cause irreversible phosphorylation of esterases in the central nervous system of insects and mammals, and act as cholinesterase inhibitors (Conteras *et al*., 1999; Rayd, 1999), which was also found to lead to the chromosomal damage associated with bladder cancer (Webster *et al*., 2002). Such concerns have heightened the need for innovative and advanced technology for effective removal of MP from a variety of contaminated environmental sources including water, sediments and soils.

Biodegradation is a common method for the removal of organic pollutants because of its low cost and little collateral destruction of indigenous flora and fauna (Timmis and Pieper, 1999). Bacterial phosphotriesterase (PTE) is an enzyme with high catalytic activity hydrolyzing a broad range of organophosphates through the cleavage of P–O and P–S bonds in these organophosphates (Ang *et al*., 2005). A few species of MP biodegrading bacteria were isolated from the environment (Wang *et al*., 2001) and the metabolic pathway of MP by different bacterial strains were also studied (Kullman and Matsumura 1996; Matys *et al*., 2001). Furthermore, the purification, characterization and expression of MP hydrolase were also investigated (Fu *et al*., 2004; Liu *et al*., 2004); however, no report on the biodegradation of MP by *Acinetobacter radioresistens* was previously found.

In this study, to effectively remove MP from the environment, special bacteria *A. radioresistens* USTB-04, as a new strain capable of biodegrading MP, was indentified, and the MP-degradation activity was investigated, which is very important in both the basic research and application in environmental sciences.

1 Materials and methods

1.1 Materials

MP was from the Institute of Environmental Monitor, Ministry of Agriculture, China. All other chemicals in the

Project supported by the National Natural Science Foundation of China (No. 20377047, 20507002) and the Innovation Foundation in University of Science and Technology Beijing. *Corresponding author. E-mail: haiyan@sas.ustb.edu.cn.

experiment were analytical grade. *A. radioresistens* USTB-04 isolated from the sediment of waste water treatment in a pesticide plant in Shandong Province of China using MP as a sole carbon source. *A. radioresistens* was positively classified by the Institute of Microbiology, Chinese Academy of Sciences, which was further verified by the analysis results of 16S rRNA sequence with Blast.

1.2 Culture medium and conditions

The basal liquid medium to culture *A. radioresistens* USTB-04 consists of 0.50 g of KCl, 0.02 g of $CaCl_2$, 1.00 g of $NaHCO_3$, 0.50 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $FeCl_3 \cdot 6H_2O$, 1.0 ml of trace elements solution (Sprenger *et al*., 2003) in 1000 ml distilled water. MP was added and initial pH of medium was adjusted to 7.0 with 1.0 mol/L NaOH or HCl. To test the effects of other compounds on the biodegradation of MP, 10.0 g/L of glucose as carbon source, 1.0 g/L of urea as nitrogen source or phosphate (K_2HPO_4) as phosphor source were included in the basal medium. Both medium and all experiment utensils were sterilized at 124°C for 20 min.

A. radioresistens USTB-04 was inoculated in the sterilized culture medium, and grown in a 100 ml flask that contained 30 ml liquid medium. The culture condition was at the temperature of 30°C and the shake rate of 200 r/min. Culture broth 1.0 ml was taken and directly extracted with 1.0 ml of *n*-hexane by shaking for 1 min on a vortex. After centrifugation at 10000 r/min for 10 min, the supernatant of *n*-hexane was diluted and used to determine the concentration of MP on high performance liquid chromatography (Shimadzu-LC10A, Japan). The data presented here were the average values derived from three measurements and their relative standard deviations were less than 10%.

1.3 Preparation of cell-free extract

The cells of *A. radioresistens* USTB-04 were harvested by centrifugation at 10000 r/min for 10 min at 4°C, followed by washing twice with 50 mmol/L potassium phosphate buffer (PB, pH 7.0). Then the cells were resuspended in 20 ml PB solution and disrupted using an Ultrasonic Cell Disruptor (JY92-2D, Ningbo New Michigan Biological Science and Technology Co. Ltd, China) with an output power of 400 W for 20 min. The cell debris was removed by centrifugation at 15000 r/min for 20 min and the supernatant was saved as the cell-free extract in MP degrading reactions. The concentration of protein was determined according to the method by Bradford using bovine serum albumin as the standard (Bradford, 1976). The reactions containing the mixture of cell-free extract and MP in PB solution were performed at the temperature of 30°C and the shake rate of 200 r/min. MP taken at various time points was extracted with *n*-hexane and measured on HPLC.

1.4 Analysis of MP

MP was measured using a HPLC system (Shimadzu LC-10ATVP, Shimadzu Co., Japan) with a UV Diode Array Detector at 271 nm connected to a SB-C18 (4.6 mm × 250 mm, 5 μm) column from Agilent. The mobile phase was 80% (v/v) methanol-water solution and the flow-rate was 1.0 ml/min. The calibration curve was established between the peak areas and the concentration of MP in *n*-hexane, which was used to calculate the unknown concentration of MP in the experiment.

2 Results and discussion

2.1 Effects of glucose, urea and phosphate on the biodegradation of MP

Fig.1 shows the effects of glucose, urea and phosphate on the biodegradation of MP by *A. radioresistens* USTB-04. Under restrictive culture condition when MP was used as the sole carbon, nitrogen and phosphorus sources, *A. radioresistens* USTB-04 can degrade MP from an initial concentration of 1200 mg/L to 700 mg/L in 4 d. However, when glucose was added to the medium at a concentration of 10.0 g/L, MP was hardly biodegraded. The biodegradation of MP was accelerated and MP could be totally removed at the day 2 and 4 in the presence of phosphate of 1.0 g/L or urea of 1.0 g/L, respectively.

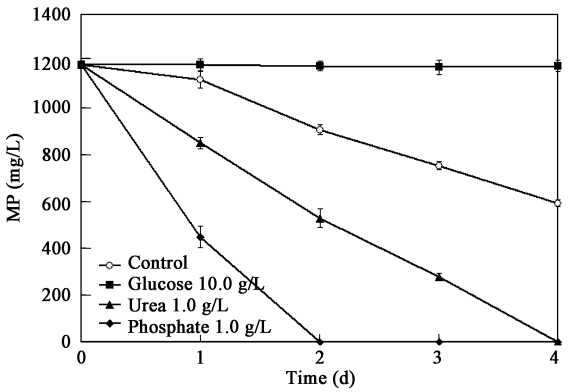

Fig. 1 Effects of glucose, urea and phosphate on the biodegradation of MP by *A. radioresistens* USTB-04.

MP contains carbon, nitrogen and phosphor elements. When MP is used by bacteria as the sole carbon, nitrogen or phosphor sources, it can theoretically be biodegraded. When glucose was present in MP-containing medium, MP could not be biodegraded, implying difficulty for *A. radioresistens* USTB-04 to use MP as the sole nitrogen or phosphor source. On the other hand, *A. radioresistens* USTB-04 degraded MP at a higher rate in the presence of urea or phosphate (Fig.1), which is in agreement with an earlier report in which MP was used as a sole carbon source (Liu *et al*., 2005). In the future it is possible to use *A. radioresistens* USTB-04 to degrade MP in a practical manner by manipulating its culture conditions.

2.2 Biodegradation of MP by the cell-free extract of *A. radioresistens* USTB-04

Figure 2 shows the biodegradation of MP by cell-free extracts containing protein at concentrations of 14.8, 74.0 and 148.0 mg/L, respectively. It indicated that the biodegradation of MP was enhanced with the augment of

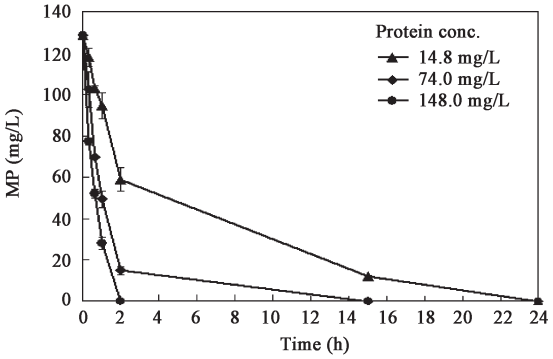

Fig. 2 Biodegradation of MP by the enzymes of *A. radioresistens* USTB-04 with different protein concentrations at 30°C and pH 7.0.

protein concentration, and initial MP of 130 mg/L was completely eliminated in 2, 15 and 24 h, respectively. It also indicated that the peak of MP on HPLC profile gradually decreased in the presence of protein of 74.0 mg/L, however, no any other intermediate metabolites or dead-end product could be observed with the different periods of reaction (Fig.3), even if gas chromatograph mass spectrum and liquid chromatograph mass spectrum were used to detect the product, respectively.

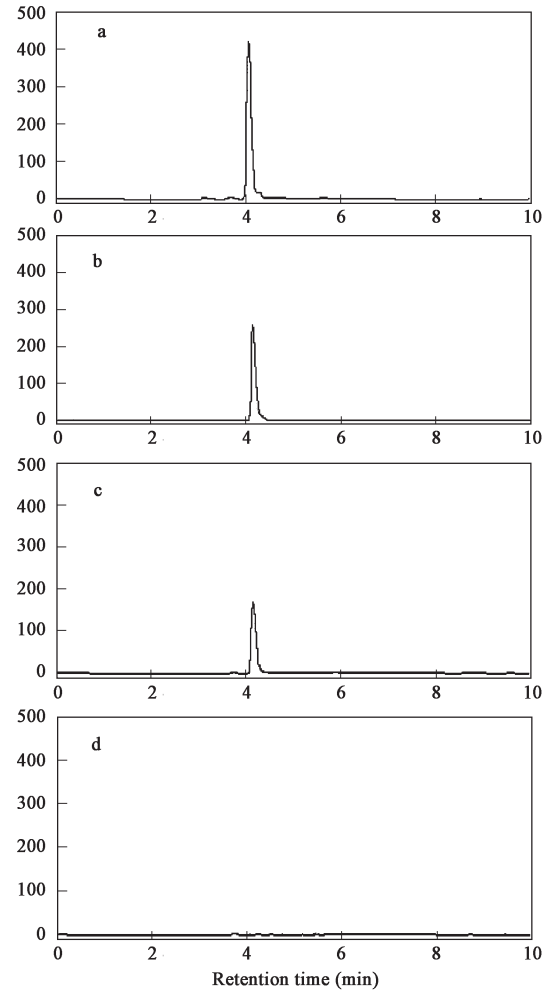

Fig. 3 HPLC profiles for the biodegradation of MP by the enzymes of *A. radioresistens* USTB-04 in different reaction periods. (a) 0 h; (b) 0.3 h; (c) 2.0 h; (d) 15.0 h.

Figure 4 shows the effects of pH on the hydrolysis and biodegradation of MP at initial MP of 100 mg/L by the cell-free extract at the protein concentration of 100 mg/L. In the absence of cell-free extract, the hydrolyzed efficiency of MP were lower than 3.0% in the pH range from 5.0 to 8.0, but increased to 27.0% with the increase of pH from 8.0 to 11.0. In the presence of cell-free extract, the elimination efficiency of MP was increased from 20% to about 90% with the augment of pH, which implied that the activity of biodegradation enzymes in *A. radioresistens* USTB-04 requires an alkaline environment.

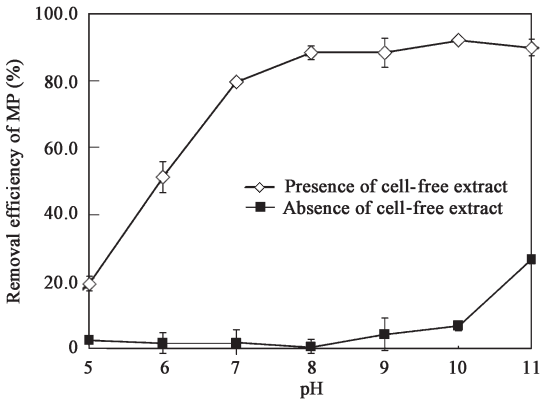

Fig. 4 Effects of pH on the biodegradation of MP by the enzymes of *A. radioresistens* USTB-04.

PTE was a highly efficient hydrolytic enzyme that catalyzed the cleavage of P–O, P–F or P–S bonds in organophosphate compounds (Ang *et al*., 2005). Enzymatic hydrolysis of MP could reduce the toxicity by nearly 120-fold and lead to the formation of *p*-nitrophenol (PNP) as a byproduct (Munnecke, 1979), which was frequently found in both terrestrial and aquatic environments (Ohshiro *et al*., 1999). PNP was also reported to be an intermediate product of MP biodegradation by two *pseudomonas* strains (Chen *et al*., 2002). In our experiment, PNP could not be detected using the HPLC during the biodegradation of MP by the enzymes of *A. radioresistens* USTB-04 (Fig.3), with the detection wavelength of 405 nm which is the maximum absorbance of PNP. Absence of PNP during MP biodegradation suggests that *A. radioresistens* USTB-04 may degrade MP by another pathway different from previous reports (Munnecke, 1979; Ohshiro *et al*., 1999).

It was reported that *A. radioresistens*, grown on phenol or benzoate as sole carbon and energy source, could degrade aromatic compounds by induction of two novel catechol 1,2-dioxygenases (Briganti *et al*., 1997; Caposio *et al*., 2002; Kim *et al*., 2003). So benzene ring rather than P–O bond of MP might be first attacked by *A. radioresistens* USTB-04. This possibility is supported by the observation that MP could be used as sole carbon but not nitrogen and phosphor sources. If the P–O bond of MP was cleaved by PTE of *A. radioresistens* USTB-04, phosphoric acid would be easily produced, which meant that MP could be used as the sole phosphor source. Since MP could not be used as the sole phosphor source by *A.*

radioresistens USTB-04 (Fig.2), the cleavage of P–O on MP is not likely to occur. Based on these observations we speculate that the cleavage of benzene ring played a key role in the biodegradation of MP by *A. radioresistens* USTB-04. Further characterization of the enzymes and genes involved in biodegradation of MP in *A. radioresistens* USTB-04 is warranted.

3 Conclusions

We identified *A. radioresistens* USTB-04 as a new strain capable of biodegrading MP under restrictive culture conditions. The biodegradation rate of MP could be increased in the presence of phosphate or urea, could be completely eliminated in 2 and 4 d, respectively, under that condition MP with an initial concentration of 1200 mg/L. A cell-free extract preparation from *A. radioresistens* USTB-04 exhibited MP-degradation activity at the optimal pH over 8.0 for the enzymatic reactions. During the reaction of MP catalyzed by the enzymes of *A. radioresistens* USTB-04, no known intermediate or dead-end product could be detected using HPLC, suggesting that the cleavage of benzene ring might play a key role in the biodegradation of MP by *A. radioresistens* USTB-04.

Acknowledgements

The authors acknowledge the financial support for this study from the National Nature Science Fundation of China and University of Science and Technology Beijing. We would like to thank Prof. Qing Song in the University of Science and Technology Beijing for the revision of this paper.

References

Ang E L, Zhao H M, Obbard J P, 2005. Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering[J]. Enz Microb Technol, 37: 487–496.

Bradford M M, 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principle of protein-dye binding[J]. Anal Biochem, 72: 248–254.

Briganti F, Pessione E, Giunta C *et al*., 1997. Purification, biochemical properties and substrate specificity of a catechol 1,2-dioxygenase from a phenol degrading *Acinetobacter radioresistens*[J]. FEBS Lett, 416: 61–64.

Caposio P, Pessione E, Giuffrida G *et al*., 2002. Cloning and characterization of two catechol 1,2-dioxygenase genes from *Acinetobacter radioresistens* S13[J]. Res Microbiol, 153: 69–74.

Chen Y L, Zhang X E, Liu H *et al*., 2002. Study on *Pseudomonas* sp. WBC-3 capable of complete degradation of methyl parathion[J]. Acta Microbiologica Sinica China, 42: 490–497.

Conteras H R, Badilla J, Bustosobregan E, 1999. Mophofunctional disturbance in human sperm cell after incubation with organophosphate pesticides[J]. Biocell, 23: 135–141.

Fu G P, Cui Z L, Huang T T *et al*., 2004. Expression, purification, and characterization of a novel methyl parathion hydrolase[J]. Protein Expres Purif, 36: 170–176.

Kim S I, Song S Y, Kim K W *et al*., 2003. Proteomic analysis of the benzoate degradation pathway in *Acinetobacter* sp. KS-1[J]. Res Microbiol, 154: 697–703.

Kullman S W, Matsumura F, 1996. Metabolic pathways utilized by *Phanerochaete chrysosporium* for degradation of the cyclodiene pesticide endosulfan[J]. Appl Environ Microbiol, 62: 593–600.

Liu Y H, Liu Y, Chen Z S *et al*., 2004. Purification and characterization of a novel organophosphorus pesticide hydrolase from *Penicillium lilacinum* BP303[J]. Enz Microb Technol, 34: 297–303.

Liu H, Zhang J J, Wang S J *et al*., 2005. Plasmid-borne catabolism of methyl parathion and *p*-nitrophenol in *Pseudomonas* sp. strain WBC-3[J]. Biochem Biophys Res Commun, 334: 1107–1114.

Matys S V, Laurinavichius K S, Krupyanko V I *et al*., 2001. Optimization of degradation of methylphosphonate-analogue of toxic pollutants with direct C–P bond by *Escherichia coli*[J]. Process Biochem, 36: 821–827.

Munnecke D W, 1979. Hydrolysis of organophosphate insecticides by an immobilized-enzyme system[J]. Biotechnol Bioeng, 21: 2247–2261.

Ohshiro K, Kakuta T, Nikaidou N *et al*., 1999. Molecular cloning and nucleotide sequencing of organophosphorus insecticide hydrolase gene from *Arthrobacter* sp. strain B-5[J]. J Biosci Bioeng, 87: 531–534.

Rayd E, 1999. Pesticide neurotoxicity in Europe: Real risks and perceived risks[J]. Neurotoxicol, 21: 219–221.

Sprenger W W, Dijkstra A, Zwart G J M *et al*., 2003. Competition of a parathion-hydrolyzing *Flavobacterium* with bacteria from ditch water in carbon-, nitrate- and phosphate-limited continuous cultures[J]. FEMS Microbiol Ecol, 43: 45–53.

Timmis K N, Pieper D H, 1999. Bacteria designed for bioremediation[J]. Trends Biotechnol, 17: 201–204.

Tsatsakis A M, Tsakiris I N, Maxaira K *et al*., 2002. Assessment of safe harvesting after methyl parathion application in peaches[J]. Bull Environ Contam Toxicol, 68: 824–830.

Wang Y J, Li S P, Shen B, 2001. Isolation and activity of dimethoate-degrading strain[J]. J Nanjing Agricul Univ China, 24: 71–74.

Webster L R, Mckenzie G H, Moriarty H T, 2002. Organophosphate-based pesticides and genetic damage implicated in bladder cancer[J]. Cancer Genet Cytogen, 133: 112–117.