Journal of Hazardous Materials 221–222 (2012) 178–184

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

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

Plasmid-mediated bioaugmentation for the degradation of chlorpyrifos in soil

Qun Zhang[a,b], Baichuan Wang[a,b], Zhengya Cao[a,b], Yunlong Yu[a,b,*]

[a] Institute of Pesticide and Environmental Toxicology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, PR China
[b] Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Ministry of Agriculture, Hangzhou 310058, PR China

ARTICLE INFO

Article history:
Received 13 January 2012
Received in revised form 29 March 2012
Accepted 11 April 2012
Available online 18 April 2012

Keywords:
Plasmid transfer
Soil
Chlorpyrifos
Green fluorescent protein (GFP)
Bioaugmentation

ABSTRACT

To overcome the poor survival and low activity of the bacteria used for bioremediation, a plasmid-mediated bioaugmentation method was investigated, which could result in a persistent capacity for the degradation of chlorpyrifos in soil. The results indicate that the pDOC plasmid could transfer into soil bacteria, including members of the *Pseudomonas* and *Staphylococcus* genera. The soil bacteria acquired the ability to degrade chlorpyrifos within 5 days of the transfer of pDOC. The efficiency of the pDOC transfer in the soil, as measured by the chlorpyrifos degradation efficiency and the most probable number (MPN) of chlorpyrifos degraders, was influenced by the soil temperature, moisture level and type. The best performance for the transfer of pDOC was observed under conditions of 30 °C and 60% water-holding capacity (WHC). The results presented in this paper show that the transfer of pDOC can enhance the degradation of chlorpyrifos in various soils, although the degradation efficiency did vary with the soil type. It may be concluded that the introduction of plasmids encoding enzymes that can degrade xenobiotics or donor strains harboring these plasmids is an alternative approach in bioaugmentation.

1. Introduction

A useful approach to enhance degradation and/or detoxification of xenobiotics in the environment is bioaugmentation which involves introduction of microorganisms capable of degrading target chemicals. However, the bacteria introduced into the contaminated sites or soils usually fail to degrade the pollutants due to the poor survival or low activity of these bacteria caused by abiotic and biotic stresses [1–7].

The genes involved in the degradation of synthetic xenobiotics are usually contained on broad host-range plasmids, such as pMCP424 for organophosphorus insecticides [8,9], pRC1 for carbaryl [10], pJP4 for 2, 4-dichlorophenoxyacetic acid (2, 4-D) [11], and pADP-1 and pEST4011 for atrazine [12,13]. These transmissible plasmids can disseminate among soil microorganisms through transformation, transduction and conjugation [14,15]. For example, Aspray et al. [16] observed the transfer of the pJP4 plasmid, which encodes enzymes that can degrade 2, 4-D, from the donor, *Pseudomonas putida* SM1443, to members of the *Burkholderia* genus, and the *Burkholderia* transconjugant was able to degrade 2, 4-D. Moreover, the *mpd* gene encoding the organophosphorus hydrolase was cloned from chlorpyrifos-degrading bacterium (strain YC-1) and expressed in *Escherichia coli* (*E. coli*), which resulted in the successful degradation of chlorpyrifos in soil by strain YC-1 [17]. The horizontal transfer of plasmids could induce the dissemination and subsequent recombination of catabolic genes in soil microbial communities and thus increase the metabolic potential of the natural microbial communities for bioremediation [18–20]. Therefore, the introduction of plasmid-borne genes for the degradation of xenobiotics into well-established and competitive indigenous bacteria might be an alternative approach to overcome the poor survival and low activity of introduced bacteria used for bioaugmentation. The introduction of a degradative plasmid donor as a method of gene bioaugmentation or plasmid-mediated bioaugmentation has been attempted [21–23]. However, most of the reports focus on the frequency of plasmid transfer in the soil, and little attention has been devoted to the important endpoints (the degradation efficiency and the size of plasmid transfer) of plasmid transfer in the soil in bioaugmentation. It is also necessary to assess the performance of plasmid transfer in bioaugmentation under various environmental conditions.

In our earlier experiments, a bacterial strain capable of degrading chlorpyrifos, *Bacillus laterosporus* DSP, was isolated from the soil for the biodegradation and/or detoxification of chlorpyrifos, and the pDOC plasmid in the DSP strain was found to be responsible for the degradation of chlorpyrifos. The main goal of the present study was to develop a plasmid-mediated bioaugmentation method that could result in the persistent capacity for the degradation of chlorpyrifos in the soil. Specific aims of this study were: (1) to introduce plasmid pDOC for bioaugmentation of chlorpyrifos in soil and

* Corresponding author at: Institute of Pesticide and Environmental Toxicology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, PR China. Tel.: +86 571 88982433; fax: +86 571 88982433.
E-mail address: ylyu@zju.edu.cn (Y.L. Yu).

0304-3894/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jhazmat.2012.04.024

monitor the transfer of pDOC to the indigenous community using GFP fluorescence; (2) isolate transconjugants using GFP fluorescence; and (3) assess the effects of key environmental factors on plasmid-mediated bioaugmentation of chlorpyrifos in soil.

2. Materials and methods

2.1. Chemicals, soils, bacterial strains and plasmids

The chemicals and soils used in this study are described in detail in Supplementary Material S1 and Table S1. The bacterial strains and plasmids used in this work and their relevant characteristics are listed in Table S2.

The pDOC plasmid from strain DSP was determined to be responsible for chlorpyrifos degradation [24]. Small- and large-scale extractions of the plasmid were performed using the AxyPrepTM Plasmid Miniprep Kit (AXYGEN Biotechnology Co., Hangzhou, China) according to the manufacturer's instructions and using the alkali lysis method [25], respectively. The obtained lysates were stored at −20 °C until use. The approximate size of the plasmid DNA was determined by comparing its mobility with that of a standard molecular marker (λDNA/HindIII (TaKaRa Biotechnology Co. Ltd., DaLian, China)) on a 1% agarose gel electrophoresis.

2.2. Construction of the gfp-tagged plasmid and its donors

The insertion of *gfp* into the pDOC plasmid was performed by triparental mating in which the *Escherichia coli* HB101 helper strain was used to mobilize the *gfp* segment from the *Escherichia coli* DH5α donor strain into the DSP recipient strain. The DSP, DH5α, and HB101 strains were incubated separately at 30 °C overnight in 5 mL of Luria-Bertani (LB; 5 g Bacto tryptone, 3 g yeast extract, 3 g NaCl, and 1 L distilled water, pH 7.0) medium, and then the cells were collected by centrifugation (8000 × *g*, 10 min), washed with sterile, double de-ionized water, re-centrifuged (8000 × *g*, 10 min), and re-suspended in 1 mL of sterile, double de-ionized water. Suspensions (200 μL) of the DSP, DH5α, and HB101 strains were mixed in a 1.5 mL sterile microcentrifuge tube, centrifuged (5000 × *g*) for 10 min and re-suspended in 1 mL LB. After incubation at 30 °C overnight, the mixture was plated onto basal medium supplemented with 50 mg/L of kanamycin sulfate (Km), spectinomycin (Sm), and chlorpyrifos and incubated at 30 °C for two days. The DSP strain (pDOC-*gfp*) was selected based on its resistance to antibiotics (Km and Sm), its ability to utilize chlorpyrifos as the sole carbon and energy sources, and the observation of GFP expression by microscopy.

The *gfp*-tagged plasmid donor strains, JM109 (pDOC-*gfp*) and *Klebsiella* sp. TZ (pDOC-*gfp*), were obtained by transformation with the pDOC-*gfp* plasmid according to the method proposed by Sambrook and Russell [25].

2.3. Degradation of chlorpyrifos by pDOC plasmid donors

To examine the ability of the plasmid donors to degrade chlorpyrifos, each donor was inoculated at a biomass of $OD_{415\,nm}$ = 0.20 into 20 mL of mineral salt medium (MSM; 0.4 g $MgSO_4{\cdot}7H_2O$, 0.002 g $FeSO_4{\cdot}7H_2O$, 0.2 g KH_2PO_4, 0.2 g $(NH4)_2SO_4$, 0.08 g $CaSO_4$, and 1 L distilled water, pH 7.0) supplemented with chlorpyrifos as the sole source of carbon and energy at level of 10 mg/L in a 100 mL flask. After 0 and 5 days, the entire culture was sampled for the determination of the chlorpyrifos concentration, as described in Supplementary Material S2. The control experiment, which did not include inoculation with the plasmid donor, was carried out under the same conditions. Each treatment was replicated three times.

2.4. Development of chlorpyrifos degradation capacity in the soil by plasmid transfer

To explore the development of the chlorpyrifos degradation capacity in the soil by plasmid transfer, Hangzhou soil (1200 g dry weight equivalent) was mixed with a solution of chlorpyrifos in acetone to reach a final concentration of 200 mg/kg of dry soil. The soil samples were left for 1 h in a laminar flow bench for solvent evaporation and subsequently mixed thoroughly using sterile plastic spoons and passed through a 2 mm mesh to ensure the uniform distribution of the added pesticide. The purified pDOC plasmid, extracted from 5 mL of the suspension of the DSP strain with cell density of 1.0×10^7 CFU/mL, was inoculated into the soil, and the soil was homogenized by sieving through a 2 mm mesh twice with the addition of the appropriate amount of sterile, distilled water for moisture content adjustment (60% WHC). The treated soil was placed in a 2 L flower pot and incubated at 25 °C in the dark. Chlorpyrifos was applied successively 22 and 37 days after the first treatment. At time intervals of 0, 7, 14, 21, 22, 29, 36, 37, 44, and 51 days, soil samples (25 g dry weight equivalent) from each treatment were collected using a soil auger (2 cm diameter) for the determination of the residual chlorpyrifos concentration, as described in Supplementary Material S2. In addition, 10 g dry weight soil was sampled for the counting of potential degraders by the method described by Alexander [26] using chlorpyrifos as the sole organic substrate. Controls without inoculation were carried out under the same conditions. Each treatment was replicated three times.

2.5. Effect of the plasmid donor, temperature, moisture, and soil type on plasmid transfer

To assess the transfer of the pDOC plasmid from various donors into indigenous bacteria, a similar experiment to confirm the degradation capacity was conducted with the plasmid donor strains DSP (pDOC-*gfp*), JM109 (pDOC-*gfp*), and TZ (pDOC-*gfp*) at an initial cell density of 1.0×10^5 CFU/g of soil. To test the influence of the temperature, soil samples treated with the JM109 (pDOC-*gfp*) strain as the donor and chlorpyrifos at level of 10 mg/kg were incubated at 20 °C, 30 °C, or 40 °C. To confirm the effect of the soil moisture, the experiment was carried out at three different soil moisture equivalents: 40%, 60%, and 80% WHC. Soils from Hangzhou (HZ), Jiaxing (JX), Xiaoshan (XS), and Jinhua (JH) were used for the determination of the effect of the soil properties. All of the treatments were replicated three times.

2.6. Microscopy analysis of bacteria harboring pDOC-gfp

To analyze the microorganisms in the soil using fluorescence, a soil sample (5 g dry weight equivalent) was weighed in a 20 mL glass tube and mixed thoroughly with 3 mL sterile, double de-ionized water on a vortex oscillator for 30 s. One drop of the suspension and two drops of fixing solution (glycerol: phosphate buffer [pH 7.0], 1:1, v/v) were placed onto a glass slide and covered with a coverslip. The fluorescence imaging was performed using a confocal laser scanning microscope (Leica DMI 6000 CS, Leica Microsystems, Wetzlar, Germany) with the Leica Application Suite Advanced Fluorescence (LASAF) program. The wavelength of excitation ranged from 500 to 530 nm.

3. Results and discussion

3.1. The pDOC plasmid and the degradation of chlorpyrifos

To determine the involvement of the plasmid-encoded genes of the DSP strain in chlorpyrifos degradation, a plasmid-eliminated DSP strain (DSP^-), a plasmid-recovered DSP^- strain (DSP^+), and a

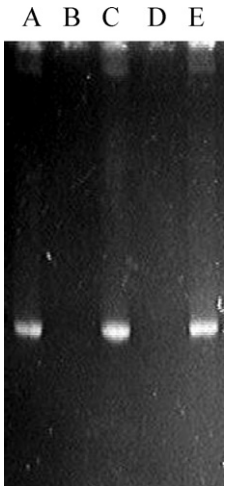

Fig. 1. Agarose gel electrophoresis of the plasmid DNA extracted from *B. laterosporus* DSP (A), *B. laterosporus* DSP$^-$ (B), *B. laterosporus* DSP$^+$ (C), *E. coli* JM109 (D), and *E. coli* JM109 (pDOC) (E).

plasmid-transformed JM109 strain (pDOC) were constructed. The plasmids extracted from the DSP$^+$ and JM109 (pDOC) strains were identical to that from the original DSP strain (Fig. 1). The degradation of chlorpyrifos in MSM by the DSP, DSP$^-$, DSP$^+$, JM109, and JM109 (pDOC) strains was assessed and is shown in Table S3. Approximately 61.8%, 49.6%, and 55.5% of the added chlorpyrifos was degraded by the DSP, DSP$^+$, and JM109 (pDOC) strains, respectively, after 5 days of incubation. In contrast, the dissipation efficiencies of chlorpyrifos by the DSP$^-$ and JM109 strains were approximately 5.5% and 4.0%, respectively, which was similar to that of the control without inocula. These results clearly indicate that the pDOC plasmid in the DSP strain was responsible for the degradation of chlorpyrifos.

3.2. Development of the degradation capacity in pDOC-inoculated soil

To assess the development of the capacity for the degradation of chlorpyrifos in soil induced by plasmid transfer, the degradation of chlorpyrifos after repeated applications to the control and pDOC-inoculated soils was monitored (Fig. 2). The concentration of chlorpyrifos in control soil increased from 161.2 mg/kg on day 14 after the first addition to 200.9 mg/kg on day 14 after third addition, indicating that chlorpyrifos accumulation in the soil with repeated

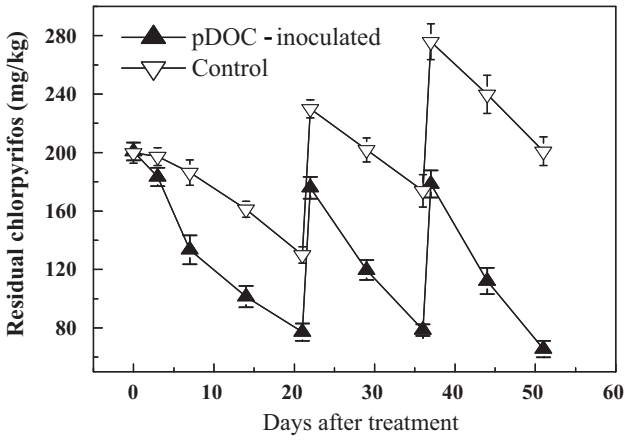

Fig. 2. Degradation of chlorpyrifos after repeated applications to the control and pDOC-inoculated soils.

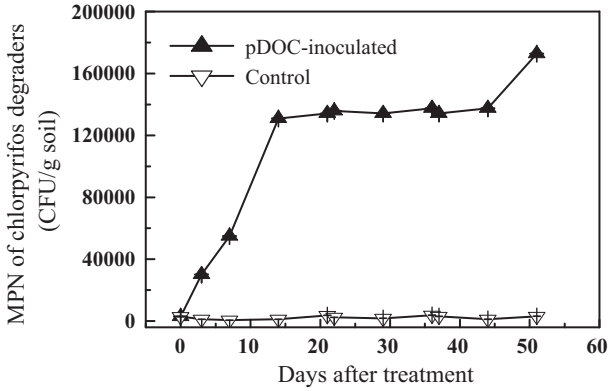

Fig. 3. MPN of chlorpyrifos degraders in soil treated with or without pDOC.

applications. On contrary, the degradation efficiency of chlorpyrifos in the pDOC-treated soil was greatly enhanced, and appeared to be accelerated after the second and third additions of chlorpyrifos. The pDOC plasmid itself is unable to degrade chlorpyrifos, and expression of the encoded gene(s) in indigenous bacteria is required for the development of degradation capacity for chlorpyrifos. Therefore, the chlorpyrifos degradation capacity in the pDOC-inoculated soil indicated that the plasmid was transferred into indigenous soil bacteria. This transfer was confirmed by the variation in the MPN of the microorganisms capable of degrading chlorpyrifos in the pDOC-treated soil (Fig. 3). The MPN in the pDOC-treated soil increased gradually from almost none (29 CFU/g of soil) on day 0 to approximately 130,952 CFU/g of soil on day 14 after the first addition of chlorpyrifos and maintained at a stable high level with the following applications of the chemical.

3.3. Plasmid pDOC transfer events

To confirm the transfer of the pDOC plasmid into indigenous soil bacteria, soil containing chlorpyrifos at 50 mg/kg was treated with pDOC-*gfp* and incubated at 30 °C in the dark. Soil samples were collected 0, 5, 10, and 30 days after the treatment for the monitoring of GFP expression by the indigenous soil bacteria. The GFP expression in the indigenous soil bacteria clearly illustrated that pDOC was transferred into the soil bacteria and that the encoded genes were expressed (Fig. 4). Fluorescent cells in the soil represent transconjugant cells carrying *gfp*-tagged pDOC because GFP is only expressed in the case of successful transfer. No fluorescent cells in the pDOC-treated soil were observed within 1 h after the treatment. The transfer of the pDOC plasmid to the soil bacteria occurred within 5 days. Haagensen et al. [27] also found that transconjugants of the TOL plasmid formed within 4 days after inoculation. Furthermore, Musovic et al. [28] found that the transfer of the pRP4-*gfp* conjugal plasmid from *Pseudomonas putida* KT2442 into the soil bacteria occurred under all test conditions. It is likely that the rapid degradation of chlorpyrifos in the pDOC-*gfp*-treated soil was induced by the transfer of pDOC into the soil bacteria.

More interestingly, four of GFP-positive strains, ZQ1, ZQ2, ZQ3, and ZQ4, were isolated from the pDOC-*gfp*-treated soil 30 days after treatment (Fig. S1) and were extracted and identified by electrophoresis (Fig. 5). By comparing the relative mobilities of the plasmids, all were found to be identical to that in the initial strain, DSP, suggesting that new chlorpyrifos degraders were formed by the transfer of pDOC. The 16S rDNA sequences of the ZQ1–4 isolates were determined and have been submitted to the NCBI GenBank databases under accession numbers JN541207, JN541208, JN541210 and JN541209, respectively. The isolates were identified as members of *Pseudomonas* (ZQ1 and ZQ2) and *Staphylococcus* (ZQ3 and ZQ4). These isolates possess the capacity to degrade

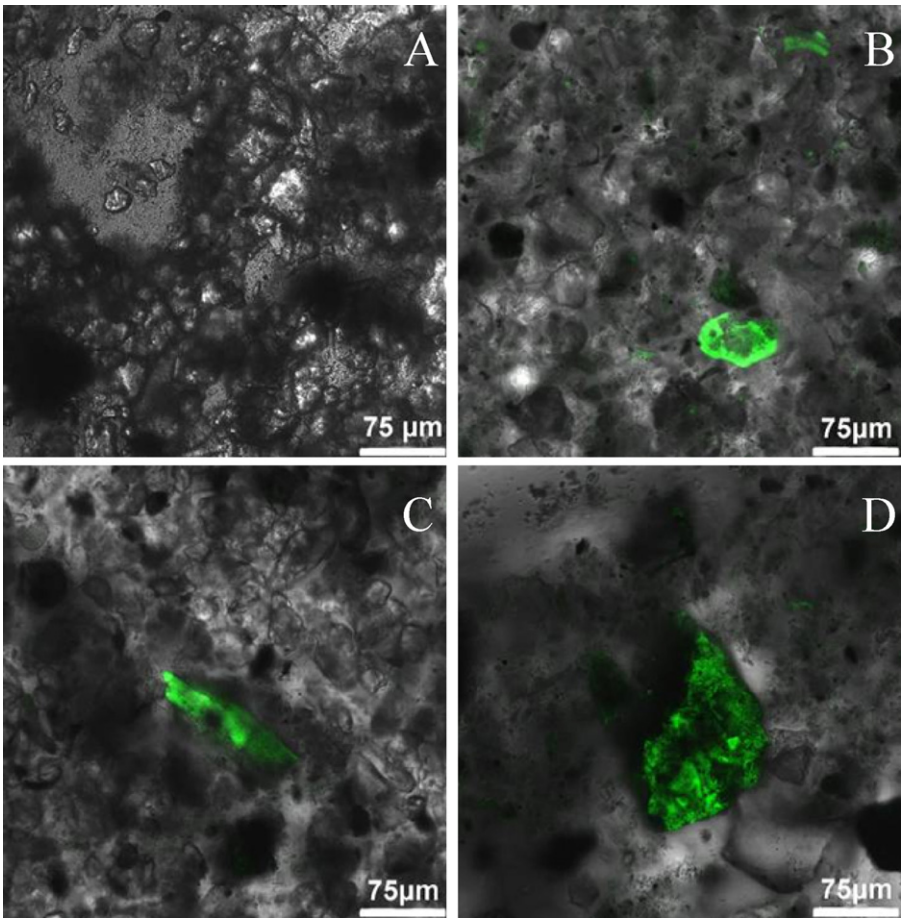

Fig. 4. GFP expression in indigenous bacteria in pDOC-*gfp*-inoculated soil 0 (A), 5 (B), 10 (C), and 30 (D) days after treatment.

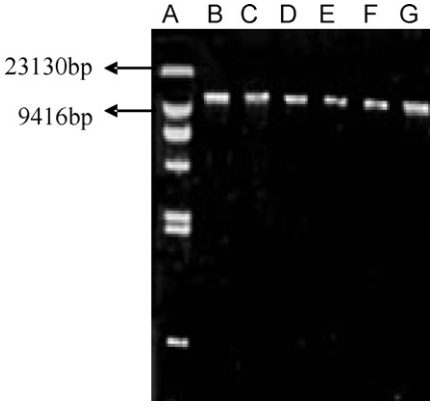

Fig. 5. Agarose gel electrophoresis of plasmid DNA. (A) λ-HindIII-digested DNA marker, (B) *Staphylococcus* sp. ZQ3, (C) *Staphylococcus* sp. ZQ4, (D) *Pseudomonas* sp. ZQ1, (E) *Pseudomonas* sp. ZQ2, (F) *B. laterosporus* DSP, and (G) *B. laterosporus* DSP (pDOC-*gfp*).

chlorpyrifos, and approximately 59.5%, 62.6%, 58.9%, and 52.2% of the added chlorpyrifos in the MSM was degraded by the ZQ1, ZQ2, ZQ3, and ZQ4 strains, respectively (Fig. S2).

3.4. Effect of pDOC donors

The transfer of a plasmid from the donor strain into other bacteria may be influenced by the type of donor. To test the influence of the donor, the DSP, DSP (pDOC-*gfp*), JM109 (pDOC-*gfp*), and TZ (pDOC-*gfp*) strains were all used as the donor to assess their performance. At 5 days after treatment, no significant difference was observed in the degradation efficiency of chlorpyrifos among the samples containing the different strains (Fig. 6). Thereafter, the degradation of chlorpyrifos in the JM109 (pDOC-*gfp*)- and TZ (pDOC-*gfp*)-treated soils appeared to be more rapid than the degradation in the DSP- and DSP (pDOC-*gfp*)-amended soils (Fig. 6). However, no significant difference was observed in the MPN of the degraders in the treated soils (Table 1). The microbial degradation efficiency of a chemical in the soil is largely governed by both the number and the activity of the microorganisms capable of

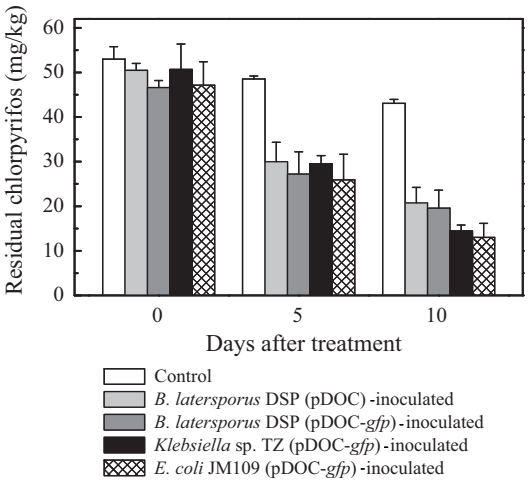

Fig. 6. Degradation of chlorpyrifos in soil treated with different pDOC donors.

Table 1
MPN of chlorpyrifos (at 50 mg/kg) degraders in soil treated with different pDOC-*gfp* plasmid donors.

Soil treatment	Chlorpyrifos degraders ($\times 10^5$ CFU/g of soil)		
	0 d	5 d	10 d
Control	ND[a]	0.03 ± 0.01	0.55 ± 0.17
B. laterosporus DSP	0.80 ± 0.40	1.30 ± 0.50	5.70 ± 2.50
B. laterosporus DSP (pDOC-*gfp*)	0.90 ± 0.54	2.00 ± 0.50	8.87 ± 3.87
E. coli JM109 (pDOC-*gfp*)	0.90 ± 0.20	2.73 ± 0.63	6.29 ± 2.37
Klebsiella sp. TZ (pDOC-*gfp*)	0.70 ± 0.30	1.36 ± 0.54	6.29 ± 1.73

[a] Not determined.

degrading the chemical. It has been reported that extrinsic bacteria would encounter severe competition with indigenous bacteria in soil [29]. Moreover, indigenous isolates would be better adapted to the soil environment for bioremediation [30]. In this study, the DSP strain was isolated from the soil collected from Cixi, Zhangjiang, China, whereas TZ was isolated from the soil used in this experiment. Thus, the strains JM109 and TZ are niche-adaptable and might be more active in the tested soil than the extrinsic strain DSP. The difference in the niche-adaptable of donor strains resulted in the difference in the degradation efficiency of chlorpyrifos in the treated soils. The results indicated that niche-adaptable donor strains are more effective than extrinsic donors in plasmid-mediated bioaugmentation.

3.5. Effect of temperature

It is probable that the temperature can affect the plasmid transfer in soil because the plasmid donor strain and soil bacteria may have different optimal temperatures for their growth and activity. The effect of the temperature on the degradation of chlorpyrifos in the JM109 (pDOC-*gfp*)-inoculated soil is shown in Fig. 7. Approximately 34.3%, 65.0%, and 56.5% of the introduced chlorpyrifos was degraded 5 days after the treatment at the temperatures of 20 °C, 30 °C, and 40 °C, respectively. The degradation efficiency of chlorpyrifos at 30 °C was much faster than those at 20 °C and 40 °C. Similarly, the efficiencies at 20 °C, 30 °C, and 40 °C determined on day 10 were 54.1%, 92.3%, and 65.9%, respectively. The MPN of the potential degraders in the soil incubated at 30 °C was found to be greater than those at 20 °C and 40 °C (Table S4). This suggested that the optimum temperature for chlorpyrifos degradation by JM109 (pDOC-*gfp*) was 30 °C. In agreement with our results, Xu et al. [31] had reported that the optimum temperature for chlorpyrifos degradation by the mixed culture of the bacterium *Serratia* sp. and the fungus *Trichosporon* sp. was 30 °C. According to Johnsen and Kroer [32], the transfer of the pRO103 plasmid increased with increasing temperature to reach a maximum at 29 °C and then decreased markedly at 35 °C. Similarly, the transfer of bla_{NDM-1} plasmids into *E. coli*, *Salmonella enterica* serotype enteritidis, and *Shigella*

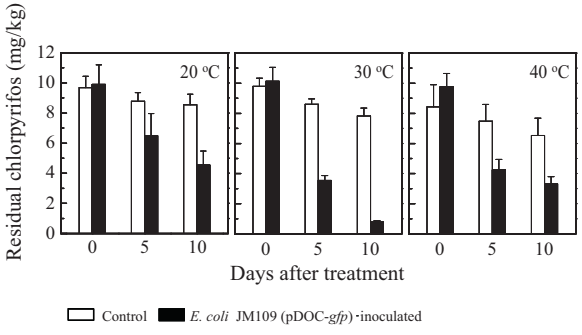

Fig. 7. Effect of temperature on the degradation of chlorpyrifos in soil with or without *E. coli* JM109 (pDOC-*gfp*)-inoculated.

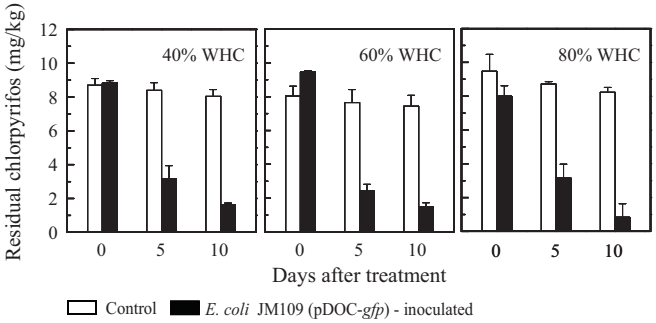

Fig. 8. Effect of soil moisture on the degradation of chlorpyrifos in soil with or without *E. coli* JM109 (pDOC-*gfp*)-inoculated.

sonnei at higher frequencies at 30 °C than at 25 °C or, particularly, at 37 °C [33]. Therefore, these results may be attributed to the optimal growth temperature of 30 °C for chlorpyrifos-degrading bacteria and the most efficient transfer of the pDOC plasmid at 30 °C. The results indicated that temperature has greatly effect on transfer frequencies and thus affect the degradation efficiencies.

3.6. Effect of moisture

Plasmid transfer from donor to recipient is governed by their direct contact, and water in the soil is the essential medium for the microbial migration by which the contact between donors and recipients takes place. The degradation of chlorpyrifos in the JM109 (pDOC-*gfp*)-inoculated soil at 60% WHC was faster than the degradation at 40% and 80% WHC (Fig. 8). Approximately 63.8%, 74.4%, and 60.5% of the added chlorpyrifos was removed within 5 days at soil moisture levels of 40%, 60%, and 80% WHC, respectively. Accordingly, the greatest MPN of the potential degraders was found at a 60% WHC, followed by 40% and 80% WHC (Table S4). The effects of moisture on the chlorpyrifos degradation and the MPN of the degraders observed on day 5 were similar to those on day 10. In general, the optimal growth of most soil microorganisms is at approximately 60% WHC [34]. The optimal growth of soil bacteria at moisture of 60% WHC promote bacterial persistence, succession, population density and thus enhance the transfer efficiencies of plasmid in soil [35]. Soil moisture is also an important factor affecting the efficiencies at which the transfer of plasmid takes place. The moisture level at 60% WHC may be more suitable for the transfer of pDOC in soil than 40% or 80% WHC.

3.7. Effect of soil type

The soil type has been found to be an important factor that influences plasmid transfer in the soil [36]. As shown in Fig. 9, the dissipation of chlorpyrifos was influenced by the soil type. After adjustment for the natural dissipation in the soil, the degradation efficiency of chlorpyrifos induced by the inoculation of the JM109 (pDOC-*gfp*) strain 5 days after treatment in the HZ soil (1.26 mg/kg d) was similar to the efficiencies in the XS (1.07 mg/kg d) and JX (1.10 mg/kg d) soils but was significantly greater than that in the JH (0.86 mg/kg d) soil. Similarly, the degradation efficiency was measured at 10 days after treatment and was 0.64, 0.86, 0.88, and 0.71 mg/kg d in the JH, HZ, XS, and JX soils, respectively. It is likely that the transfer of pDOC in the JH soil is poorer than that in the other soils because the measured degradation efficiency directly reflects the efficiency of the plasmid transfer in the soil.

The MPN values in the JM109 (pDOC-*gfp*)-treated soils are given in Table S4. The numbers of potential chlorpyrifos degraders measured both on day 5 and 10 in the HZ and XS soils were greater

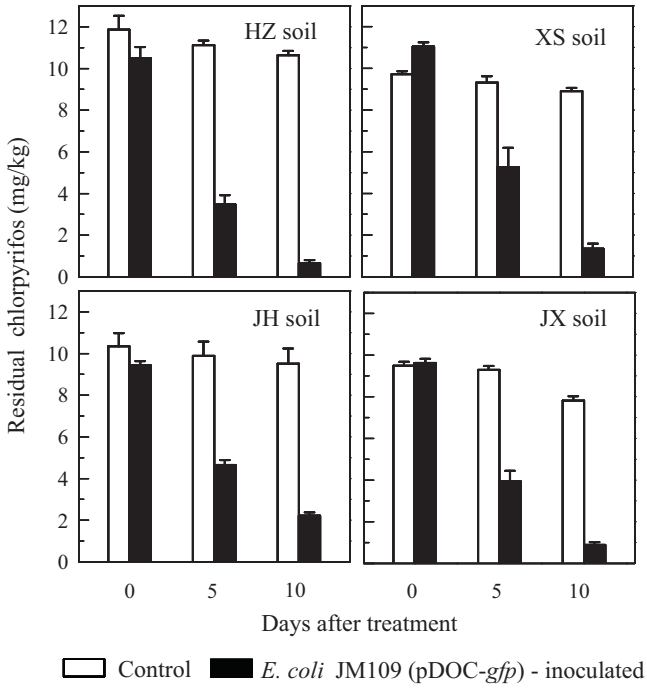

Fig. 9. Effect of soil types on the degradation of chlorpyrifos in soil with or without *E. coli* JM109 (pDOC-*gfp*)-inoculated.

than those in the JX and JH soils. No clear relationship between the MPN values, the degradation efficiency of chlorpyrifos, and the soil characteristics was observed due to the following reasons: (1) the transfer of a plasmid among soil bacteria is governed by the soil physico-chemical characteristics and the bacterial composition and populations [37]; (2) the microbial degradation of a chemical in the soil is affected by the number of degraders and their activity [38,39]; (3) the bioavailability of a chemical in the soil to degraders varies with the soil type [40,41]; and (4) the MPN value measured in this study does not represent the entire community of degraders because most soil microorganisms are unculturable [42]. Nevertheless, the results obtained in this study indicate that the transfer of pDOC could occur in all of the soils used and, thus, enhanced the chlorpyrifos degradation in the soil.

4. Conclusions

In summary, the results obtained in this study indicate that the pDOC plasmid may effectively be transferred into indigenous bacteria in the soil, and the chlorpyrifos degradation capacity developed after the transfer of the pDOC plasmid. Although the efficiency is affected by such environmental conditions as the temperature and moisture, the introduction of the pDOC donor strain into the soil may be an effective approach to enhance the degradation of chlorpyrifos in soils of various types. It may be concluded that the introduction of a degradative plasmid or its donor into the soil is a useful bioaugmentation strategy for the bioremediation of organic contamination because the soil degradation capacity could persist once the plasmid is transferred into the soil bacteria.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (21177111, 20877068), the Special Fund for Environmental Scientific Research in the Public Interest (201109018), and the Major State Basic Research Development Program of China (2009CB119006).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2012.04.024.

References

[1] M. Tyagi, M.M.R. da Fonseca, C.C.C.R. de Carvalho, Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes, Biodegradation 22 (2011) 231–241.
[2] P.A. Karas, C. Perruchon, K. Exarhou, C. Ehaliotis, D.G. Karpouzas, Potential for bioremediation of agro-industrial effluents with high loads of pesticides by selected fungi, Biodegradation 22 (2011) 215–228.
[3] A.C. Morán, A. Müller, M. Manzano, B. González, Simazine treatment history determines a significant herbicide degradation potential in soils that is not improved by bioaugmentation with *Pseudomonas* sp ADP, J. Appl. Microbiol. 101 (2006) 26–35.
[4] T. Bouchez, D. Patureau, P. Dabert, S. Juretschko, J. Doré, P. Delgenès, R. Moletta, M. Wagner, Ecological study of a bioaugmentation failure, Environ. Microbiol. 2 (2000) 179–190.
[5] L. Wittebolle, N. Boon, B. Vanparys, K. Heylen, P. De Vos, W. Verstraete, Failure of the ammonia oxidation process in two pharmaceutical wastewater treatment plants is linked to shifts in the bacterial communities, J. Appl. Microbiol. 99 (2005) 997–1006.
[6] D.K. Singh, Biodegradation and bioremediation of pesticide in soil: concept, method and recent developments, Indian J. Microbiol. 48 (2008) 35–40.
[7] W. Dejonghe, J. Goris, S. El Fantroussi, M. Höfte, P. De Vos, W. Verstraete, E.M. Top, Effect of dissemination of 2, 4-dichlorophenoxyacetic acid (2, 4-D) degradation plasmids on 2, 4-D degradation and on bacterial community structure in two different soil horizons, Appl. Environ. Microbiol. 66 (2000) 3297–3304.
[8] I. Horne, T.D. Sutherland, R.L. Harcourt, R.J. Russell, J.G. Oakeshott, Identification of an *opd* (organophosphate degradation) gene in an *Agrobacterium* isolate, Appl. Environ. Microbiol. 68 (2002) 3371–3376.
[9] B.J. Bhadbhade, P.K. Dhakephalkar, S.S. Sarnaik, P.P. Kanekar, Plasmid-associated biodegradation of an organophosphorus pesticide monocrotophos, by *Pseudomonas mendocina*, Biotechnol. Lett. 24 (2002) 647–650.
[10] M. Hashimoto, A. Mizutani, K. Tago, M. Ohnishi-Kameyama, T. Shimojo, M. Hayatsu, Cloning and nucleotide sequence of carbaryl hydrolase gene (*cahA*) from *Arthrobacter* sp RC100, J. Biosci. Bioeng. 101 (2006) 410–414.
[11] N. Trefault, R. De la Iglesia, A.M. Molina, M. Manzano, T. Ledger, D. Pérez-Pantoja, M.A. Sanchez, M. Stuardo, B. González, Genetic organization of the catabolic plasmid pJP4 from *Ralstonia eutropha* JMP134 (pJP4) reveals mechanisms of adaptation to chloroaromatic pollutants and evolution of specialized chloroaromatic degradation pathways, Environ. Microbiol. 6 (2004) 655–668.
[12] J. Aislabie, A.K. Bej, J. Ryburn, N. Lloyd, A. Wilkins, Characterization of *Arthrobacter nicotinovorans* HIM, an atrazine-degrading bacterium, from agricultural soil New Zealand, FEMS Microbiol. Ecol. 52 (2005) 279–286.
[13] S. Rousseaux, G. Soulas, A. Hartmann, Plasmid localisation of atrazine-degrading genes in newly described *Chelatobacter* and *Arthrobacter* strains, FEMS Microbiol. Ecol. 41 (2002) 69–75.
[14] H. Ochman, J.G. Lawrence, E.A. Groisman, Lateral gene transfer and the nature of bacterial innovation, Nature 405 (2000) 299–304.
[15] D. Springael, E.M. Top, Horizontal gene transfer and microbial adaptation to xenobiotics: new types of mobile genetic elements and lessons from ecological studies, Trends Microbiol. 12 (2004) 53–58.
[16] T.J. Aspray, S.K. Hansen, R.G. Burns, A soil-based microbial biofilm exposed to 2,4-D: bacterial community development and establishment of conjugative plasmid pJP4, FEMS Microbiol. Ecol. 54 (2005) 317–327.
[17] C. Yang, N. Liu, X. Guo, C. Qiao, Cloning of *mpd* gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil, FEMS Microbiol. Lett. 265 (2006) 118–125.
[18] S.J. Wu, Z.H. Hu, L.L. Zhang, X. Yu, J.M. Chen, A novel dichloromethane-degrading *Lysinibacillus sphaericus* strain wh22 and its degradative plasmid, Appl. Microbiol. Biotechnol. 82 (2009) 731–740.
[19] M. Devers, N. El Azhari, N.U. Kolic, F. Martin-Laurent, Detection and organization of atrazine-degrading genetic potential of seventeen bacterial isolates belonging to divergent taxa indicate a recent common origin of their catabolic functions, FEMS Microbiol. Lett. 273 (2007) 78–86.
[20] A. Dechesne, C. Pallud, F. Bertolla, G.L. Grundmann, Impact of the microscale distribution of a *Pseudomonas* strain introduced into soil on potential contacts with indigenous bacteria, Appl. Environ. Microbiol. 71 (2005) 8123–8131.
[21] C.M. Jung, F.H. Crocker, J.O. Eberly, K.J. Indest, Horizontal gene transfer (HGT) as a mechanism of disseminating RDX-degrading activity among Actinomycete bacteria, J. Appl. Microbiol. 110 (2011) 1449–1459.
[22] M. Shintani, Y. Takahashi, H. Yamane, H. Nojiri, The behavior and significance of degradative plasmids belonging to Inc groups in *Pseudomonas* within natural environments and microcosms, Microbes Environ. 25 (2010) 253–265.
[23] S.V. Mohan, C. Falkentoft, Y.V. Nancharaiah, B.S.M. Sturm, P. Wattiau, P.A. Wilderer, S. Wuertz, M. Hausner, Bioaugmentation of microbial communities in laboratory and pilot scale sequencing batch biofilm reactors using the TOL plasmid, Bioresour. Technol. 100 (2009) 1746–1753.

[24] X. Wang, X.Q. Chu, H. Fang, J. Chen, F.M. Song, Y.L. Yu, Degradation characteristics and functions of chlorpyrifos degradation bacterium *Bacillus latersporus* DSP, Acta Pedol. Sin. 43 (2006) 648–654.
[25] J. Sambrook, D. Russell, Molecular Cloning: A Laboratory Manual, third ed., Cold Spring Harbor Laboratory Press, New York, 2001.
[26] M. Alexander, Chemical and Microbiological Properties, second ed., American Society of Agronomy Press, Madison, WI, 1982.
[27] J.A.J. Haagensen, S.K. Hansen, T. Johansen, S. Molin, In situ detection of horizontal transfer of mobile genetic elements, FEMS Microbiol. Ecol. 42 (2002) 261–268.
[28] S. Musovic, A. Dechesne, J. Sørensen, B.F. Smets, Novel assay to assess permissiveness of a soil microbial community toward receipt of mobile genetic elements, Appl. Environ. Microbiol. 76 (2010) 4813–4818.
[29] A. Ueno, Y. Ito, I. Yumoto, H. Okuyama, Isolation and characterization of bacteria from soil contaminated with diesel oil and the possible use of these in autochthonous bioaugmentation, World J. Microbiol. Biotechnol. 23 (2007) 1739–1745.
[30] K.S. Rahman, T.J. Rahman, Y. Kourkoutas, I. Petsas, R. Marchant, I.M. Banat, Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients, Bioresour. Technol. 90 (2003) 159–168.
[31] G. Xu, Y. Li, W. Zheng, X. Peng, W. Li, Y. Yan, Mineralization of chlorpyrifos by coculture of *Serratia* and *Trichosporon* spp., Biotechnol. Lett. 29 (2007) 1469–1473.
[32] A.R. Johnsen, N. Kroer, Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events, FEMS Microbiol. Ecol. 59 (2007) 718–728.
[33] T.R. Walsh, J. Weeks, D.M. Livermore, M.A. Toleman, Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study, Lancet Infect. Dis. 11 (2011) 355–362.
[34] M. Alexander, Introduction to Soil Microbiology, second ed., John Wiley and Sons, New York, 1977.
[35] K.E. Ashelford, S.J. Norris, J.C. Fry, M.J. Bailey, M.J. Day, Seasonal population dynamics and interactions of competing bacteriophages and their host in the rhizosphere, Appl. Environ. Microbiol. 66 (2000) 4193–4199.
[36] H. Heuer, K. Smalla, Manure and sulfadiazine synergistically increased bacterial antibiotic resistance in soil over at least two months, Environ. Microbiol. 9 (2007) 657–666.
[37] G.C. Ding, H. Heuer, S. Zühlke, M. Spiteller, G.J. Pronk, K. Heister, I. Kögel-Knabner, K. Smalla, Soil type-dependent responses to phenanthrene as revealed by determining the diversity and abundance of polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes by using a novel PCR detection system, Appl. Environ. Microbiol. 76 (2010) 4765–4771.
[38] B. Mertens, N. Boon, W. Verstraete, Stereospecific effect of hexachlorocyclohexane on activity and structure of soil methanotrophic communities, Environ. Microbiol. 7 (2005) 660–669.
[39] C. Poll, H. Pagel, M. Devers-Lamrani, F. Martin-Laurent, J. Ingwersen, T. Streck, E. Kandeler, Regulation of bacterial and fungal MCPA degradation at the soil–litter interface, Soil Biol. Biochem. 42 (2010) 1879–1887.
[40] H.I. Atagana, R.J. Haynes, F.M. Wallis, Optimization of soil physical and chemical conditions for the bioremediation of creosote-contaminated soil, Biodegradation 14 (2003) 297–307.
[41] D.T. Newby, K.L. Josephson, I.L. Pepper, Detection and characterization of plasmid pJP4 transfer to indigenous soil bacteria, Appl. Environ. Microbiol. 66 (2000) 290–296.
[42] P.H. Janssen, P.S. Yates, B.E. Grinton, P.M. Taylor, M. Sait, Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*, Appl. Environ. Microbiol. 68 (2002) 2391–2396.