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Bioaugmentation of DDT-contaminated soil by dissemination of the catabolic plasmid pDOD

Chunming Gao, Xiangxiang Jin, Jingbei Ren, Hua Fang*, Yunlong Yu*

Institute of Pesticide and Environmental Toxicology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China

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ABSTRACT

A plasmid transfer-mediated bioaugmentation method for the enhancement of dichlorodiphenyltrichloroethane (DDT) degradation in soil was developed using the catabolic plasmid pDOD from Sphingobacterium sp. D-6. The pDOD plasmid could be transferred to soil bacteria, such as members of Cellulomonas, to form DDT degraders and thus accelerate DDT degradation. The transfer efficiency of pDOD was affected by the donor, temperature, moisture, and soil type. Approximately 50.7% of the DDT in the contaminated field was removed 210 days after the application of Escherichia coli TG I (pDOD-gfp). The results suggested that seeding pDOD into soil is an effective bioaugmentation method for enhancing the degradation of DDT.

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Introduction

Bacteria that are capable of degrading target compounds are frequently introduced into contaminated soil to detoxify recalcitrant pollutants. However, these bioaugmentation efforts are often hampered by a rapid decrease in introduced organism numbers and limited inoculum dispersal in the contaminated zone (Pepper et al., 2002; Quan et al., 2010). Repeated introduction of the degraders and the regulation of ecological parameters such as the water and oxygen concentrations, redox potential, nutrients, pH, and temperature are usually employed to maintain the number and activity of the degraders in the contaminated soil and thus to optimize biodegradation. If there is a way developed to successfully maintain the levels of recalcitrant chemical degraders, it is possible to use bioaugmentation more effectively.

It is well known that the capacity of microbially mediated degradation of organic pollutants has been evolved because of the selective pressures imposed by naturally occurring compounds (Copley, 2009). Bacteria can obtain new catabolic functions by acquiring genes encoding organic pollutant-degrading enzymes from distantly related organisms that can metabolize the target compounds (Wiedenbeck and Cohan, 2011). Genes for the degradation of xenobiotics are frequently found on broad-host-range plasmids, such as pV2 for dichlorvos (Tang et al., 2009), pVAG33 for gamma-hexachlorocyclohexane (γ-HCH) (Zhang et al., 2010), pADP1 for atrazine (Devers et al., 2007), pJP4 for 2,4-dichlorophenoxyacetic acid (2,4-D) (Inoue et al., 2012) and pDOC for chlorpyrifos (Zhang et al., 2012). Plasmid transfer can take place between soil bacteria and has been regarded as a significant pathway for the dissemination of catabolic functions within soil microbial communities (Mohan et al., 2009; Mrozik and Piotrowska-Seget, 2010). Miyazaki et al. (2006) reported that the dissemination of plasmid-carrying catabolic genes enhanced the degradation of γ-HCH in the natural environment. Filonov et al. (2010) reported that the transfer and dissemination of a naphthalene-degrading plasmid in microbial populations accelerated naphthalene biodegradation in soil. Several studies have also revealed that plasmid transfer among bacterial populations obviously enhances the degradation of 2,4-D, benzyl alcohol, and 3-chloroaniline in biofilm reactors, sequencing batch reactors, and activated sludge reactors (Bathe et al., 2009; Mohan et al., 2009; Quan et al., 2010). Therefore, seeding non-recombinant catabolic plasmids into soil may be an effective bioaugmentation method for enhancing the degradation potential of soil microbial communities lacking the necessary...
functions for the rapid degradation of xenobiotic compounds. Moreover, this plasmid-mediated bioaugmentation might be more effective than those achieved by isolated strains, as the recipient bacteria of the seeded plasmids are indigenous and have already adapted to the environment to be bioremediated.

Because the bioremediation of recalcitrant dichlorodiphenyltrichloroethane (DDT) is a time-consuming process, the objective of this study was to identify a plasmid-mediated method for the successful bioaugmentation of DDT-contaminated soil. *Sphingobacterium* sp. D-6, which harbors a plasmid encoding genes for the degradation of DDT, was used as the source strain. The plasmid was marked with GFP to allow the plasmid transfer in the soil to be monitored. The plasmid transfer in soil, its concomitant conferment of degradative capacity toward DDT, and the application feasibility of plasmid-mediated bioaugmentation in DDT-contaminated soil were investigated in this study.

1. Materials and methods

1.1. Chemicals and soils

*p′*-DDT (purity ≥ 99.5%), kanamycin sulfate, and spectinomycin were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Acetone and n-hexane of analytical grade were purchased from Dafang Chemical Co., Hangzhou, China. The soils used in this study were collected from Hangzhou (HZ), Xiaoshan (XS), Jiaxing (JX), and Jinghua (JH), Zhejiang, China, and their characteristics are summarized in Table S1. The soil samples were dried at room temperature and sieved (2 mm) to remove stones and debris. No specific permissions were required for these soil samples and field studies. The soil moisture was adjusted to 60% of water holding capacity (WHC) and was maintained by the periodic addition of sterile water. Each treatment was replicated three times.

1.2. Bacterial strains and plasmids

The strain *Sphingobacterium* sp. D-6 (NCBI GenBank 16S rDNA accession number EU927288), which harbors a plasmid and was isolated from DDT-contaminated soil (Fang et al., 2010), was resistant to both kanamycin sulfate and spectinomycin. The strain *Klebsiella* sp. TZ, isolated from soil HZ, and the *Escherichia coli* TG I strain, purchased from Invitrogen Life Technologies, Shanghai, China, were used as the recipient strains for the construction of plasmid donors for the plasmid transfer experiments in soil. The spectinomycin-resistant strain *E. coli* DH5α (pZP201-gfp) and the kanamycin-resistant strain *E. coli* HB101 (pRK2013) were kindly provided by Prof. Shunpeng Li, Nanjing Agricultural University, Jiangsu, China.

1.3. Plasmid from strain D-6 responsible for DDT degradation

Plasmid extraction from a culture of strain D-6 was conducted using the AxyPrep™ Plasmid Miniprep Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer’s instructions. The size of the extracted plasmid DNA was estimated by comparing its electrophoretic mobility with that of a standard molecular marker (λDNA/HindIII, Fermentas Life Sciences, Burlington, ON, Canada) on a 1% agarose gel. The data showed the existence of a plasmid (designated pDOD) of approximately 20 kb in strain D-6.

To assess whether the pDOD plasmid was responsible for the degradation of DDT by strain D-6, a plasmid-eliminated strain (D-6) and a plasmid-complemented D-6′ strain (D-6′) were constructed using the procedures described by Ghosh et al. (2000). *E. coli TG I* (pDOD) and *Klebsiella* TZ (pDOD) were also constructed by transformation using the method proposed by Sambrook and Russell (2001).

To estimate the ability of the constructed strains to degrade DDT, each strain was separately inoculated (at an OD415 nm of 0.70) into 20 mL of mineral salt medium (MSM) (MgSO4·7H2O, 0.4 g; FeSO4·7H2O, 0.002 g; KH2PO4, 0.2 g; (NH4)2SO4, 0.2 g; CaSO4, 0.08 g; distilled water, 1 L; pH 7.0) supplemented with *p′*-DDT (10 mg/L) as the sole source of carbon and energy in a 100 mL flask and incubated darkly at 30 °C on a rotational shaker. At 0 and 21 days, each culture was sampled for the determination of *p′*-DDT using the method proposed by Fang et al. (2010). A control experiment without inoculation was performed under the same conditions. Each treatment was replicated three times.

1.4. Construction of gfp-tagged pDOD and its donors

The construction of gfp-tagged pDOD was achieved by triparental mating in which the helper strain, *E. coli* HB101, was used to mobilize a gfp segment from the donor strain, *E. coli DH5α*, into the recipient strain, D-6, using the method described by Zhang et al. (2012). The GFP-marked strain D-6 (pDOD-gfp) was selected according to its resistance to both kanamycin sulfate and spectinomycin, its ability to degrade DDT, and microscopic observations of GFP expression. The gfp-tagged plasmid donors strains, *Klebsiella* sp. TZ (pDOD-gfp) and *E. coli TG I* (pDOD-gfp), were obtained by transformation with the pDOD-gfp plasmid according to the method proposed by Sambrook and Russell (2001). The ability of the pDOD donors to degrade DDT was assessed by the method described above.

1.5. Transfer of pDOD to indigenous bacteria and development of DDT degradation capacity

To explore the possibility of pDOD plasmid transfer to indigenous bacteria and its resulting conferral of DDT-degradation capacity, pDOD-gfp was extracted by the alkaline lysis method (Sambrook and Russell, 2001) and inoculated into soil (1.0 kg dry weight) supplemented with 1 mg/L of *p′*-DDT. The soil was mixed thoroughly to achieve uniform distribution. The soil moisture was adjusted to 60% of water holding capacity (WHC) and was maintained by the periodic addition of sterile water. The treated soil sample was incubated at 30 °C in the dark. After 0, 15, 30, 60, 90, and 120 days, soil samples (30 g) were collected to determine the residual *p′*-DDT, to estimate the number of DDT degraders by the method described by Alexander (1982), and to observe the fluorescence intensity.

1.6. Effects of the donor, temperature, moisture and soil type on the transfer of pDOD in soil

To assess the effect of the donor strain on the transfer of pDOD to indigenous bacteria, D-6 (pDOD-gfp), *Klebsiella* sp. TZ (pDOD-gfp), and *E. coli TG I* (pDOD-gfp) were separately
inoculated at 10^5 colony forming units (CFU)/g dry soil into soil HZ (1.0 kg dry weight) supplemented with 1 mg/L of p.p'-DDT. The inoculated soil was sieved (2 mm) to achieve a uniform distribution of the donor before being transferred into a flowerpot and covered with aluminum foil containing five pinholes. All treated soils were incubated at 30 °C in the dark. After 0, 15, 30, 60, 90, and 120 days, soil samples (30 g) were collected to determine the residual p.p'-DDT, to estimate the number of DDT degraders, and to observe the fluorescence intensity. All treatments were conducted in triplicate. The influence of temperature was examined at 20, 30, and 40 °C with E. coli TG I (pDOD-gfp) as the donor. The experiment was performed at 40%, 60%, and 80% WHC to evaluate the effect of soil moisture. The soils collected from HZ, JX, XS, and JH were used to analyze the effects of the soil properties.

1.7. Field application of pDOD plasmid-mediated bioaugmentation

To explore the feasibility of using plasmid-mediated bioaugmentation to enhance the degradation of residual DDT in contaminated soil, a field located in Cixi, Zhejiang, China and contaminated by DDT from extensive and indiscriminate use prior to its ban in China was selected for the experiment. Each plot in the experimental site (60 m², 10 m²/plot) received 100 mL of TG I (pDOD-gfp) at a density of 5 × 10^10 CFU/mL by spraying with 4000 mL of distilled water. The control plots were sprayed with the same amount of distilled water without the donor. All treatments were performed in triplicate. At 0, 15, 30, 60, 90, 120, 150, 180, and 210 days after inoculation, soil samples (30 g) were collected to determine the residual DDT, to estimate the number of DDT degraders, and to observe the fluorescence intensity.

1.8. Microscopic monitoring of bacteria harboring pDOD-gfp

The soil samples (5 g dry weight) were weighed into 20-mL glass tubes containing 3 mL of sterile water and then shaken with a vortex oscillator for 1 min. Subsequently, one drop of soil suspension and two drops of fixing solution (1:1 V/V glycerol:phosphate buffer, pH 7.0) were successively collected with a pipette, dispensed onto the center of a glass slide, and then covered with a glass coverslip. A 489-nm Ar laser line and a 508-nm emission bandwidth were used for excitation and detection, respectively. The fluorescence intensity of GFP expression in bacteria harboring pDOD-gfp was visualized using confocal laser scanning microscopy (CLSM, Leica Microsystems, Germany).

1.9. Statistical analysis

The averages and standard deviations of all data were processed using Microsoft Excel 2007 (Microsoft, Redmond, Washington, USA). The difference of DDT degradation between inoculated soil and control soil were analyzed by one-way ANOVA using SPSS version 15.0 (SPSS Inc., Chicago, Illinois, USA).

![Agarose gel electrophoresis of the plasmid DNA extracted from different strains. Lanes A–G show D-6, plasmid-eliminated strain D-6−, plasmid-complemented strain D-6+, TG I, TG I (pDOD), TZ, and TZ (pDOD), respectively. Mark: λDNA/HindIII. D-6: Sphingobacterium D-6; TG I: Escherichia coli TG I; TZ: Klebsiella TZ; pDOD: plasmid for the degradation of DDT.](image1)

![Degradation of DDT in pDOD-inoculated soil. The points marked by different letters at the same treatment time between pDOD-inoculated soil and control are significantly different (p ≤ 0.05). pDOD: plasmid for the degradation of DDT.](image2)
2. Results and discussion

2.1. pDOD plasmid and DDT degradation by Sphingobacterium sp. D-6

Analysis by agarose gel electrophoresis revealed a plasmid of approximately 20 kb present in Sphingobacterium D-6 (Fig. 1). To determine the role of the pDOD plasmid in DDT degradation by strain D-6, a plasmid-cured strain (D-6−), a plasmid-complemented strain (D-6+) and plasmid-transformed E. coli TG I (pDOD) and Klebsiella TZ (pDOD) were constructed. As shown in Fig. S1, approximately 48.7%, 21.0%, 25.0%, and 24.3% of p,p′-DDT in MSM was degraded by the D-6, D-6+, TG I (pDOD), and TZ (pDOD) strains, respectively, after incubation for 21 days. However, the removal rates of p,p′-DDT in MSM containing D-6+ or untransformed E. coli TG I or Klebsiella TZ were similar to the control without inoculation. These results suggested that the degradation genes of DDT by Sphingobacterium sp. D-6 were located on the pDOD plasmid.

2.2. Conferral of degradation capability in soil from the transfer of pDOD to indigenous bacteria

Fig. 2 shows the degradation of p,p′-DDT in pDOD-gfp-inoculated soil. It can be clearly observed that inoculation with the pDOD plasmid significantly enhanced the degradation of p,p′-DDT in the soil. After incubation for 120 days, approximately 66.6% of the added p,p′-DDT had been degraded in the pDOD-inoculated soil, whereas only approximately 8.7% had been removed in the control.

To understand the mechanisms by which the degradation of p,p′-DDT in soil was accelerated by inoculation with pDOD, the most probable number (MPN) of degraders in the soil and the expression levels of pDOD-gfp in the soil bacteria were detected in parallel. The MPN of degraders in the pDOD-gfp inoculated soil increased dramatically, from undetectable on day 0 to 65 × 10⁷ CFU/g dry soil on day 60, and thereafter remained at a high level, ranging from 35.0 × 10⁷ to 35.5 × 10⁷ CFU/g (Table 1). In agreement with the variation in the number of degraders with time after the inoculation of the pDOD-gfp plasmid, the fluorescence intensity of the soil suspension, which reflected the GFP expression by the indigenous bacteria, was successively enhanced from day 15 to day 120 (Fig. 3). Throughout the duration of the experiment, no DDT degraders or fluorescence were detected in the control soil without pDOD-gfp.

Table 1 – MPN of DDT degraders in pDOD-inoculated soil (×10⁷ CFU/g dry soil).

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Control</th>
<th>pDOD-gfp</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>5.5 ± 3.5</td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>8.3 ± 4.6</td>
</tr>
<tr>
<td>60</td>
<td>ND</td>
<td>65.0 ± 17.3</td>
</tr>
<tr>
<td>90</td>
<td>ND</td>
<td>35.0 ± 9.3</td>
</tr>
<tr>
<td>120</td>
<td>ND</td>
<td>35.5 ± 6.3</td>
</tr>
</tbody>
</table>

MPN: most probable number; DDT: dichlorodiphenyltrichloroethane; CFU: colony forming unit; ND: not detectable.

It is well known that plasmids, as DNA molecules, cannot directly degrade a chemical or exhibit fluorescence apart from its expression system. Therefore, the function of pDOD-gfp in

Fig. 3 – Dynamic monitoring of GFP expression by indigenous soil bacteria in pDOD-gfp-inoculated soil. A-F represent 0, 15, 30, 60, 90, and 120 days after pDOD-gfp inoculation, respectively. GFP: green fluorescent protein; pDOD-gfp: gfp-tagged plasmid for the degradation of DDT.
DDT degradation and GFP fluorescence can only be detected in the case of its successful transfer to bacteria (Heuer and Smalla, 2012). The observed fluorescence of the soil suspensions indicated that pDOD-gfp was transferred to the indigenous soil bacteria and it was well expressed. The bacteria that acquired pDOD-gfp became degraders of DDT and thus increased the number of DDT degraders in the soil. As a result of pDOD-gfp transfer, one new fluorescent transconjugant, ZYG2, was isolated from the pDOD-gfp-inoculated soil (Fig. S2). The strain ZYG2 (NCBI GenBank 16S rDNA accession number KF537627) was classified as a member of the genus Cellulomonas by morphology and 16S rDNA sequence analysis, and it was able to degrade 25.8% of the added p,p′-DDT (10 mg/L) in MSM within 21 days.

The observed data clearly indicated that the plasmid pDOD could be effectively transferred to indigenous bacteria to produce new DDT degraders and thus enhance DDT degradation in soil. Similarly, Tsutsui et al. (2010) and Inoue et al. (2012) reported that plasmid pJP4, encoding genes for the degradation of 2,4-D, could be transferred to Pseudomonas in activated sludge and to Burkholderia in soil. Mølbak et al. (2007) demonstrated that the plasmids pKJK5 and pWW0 could also be transferred from Pseudomonas putida to indigenous bacteria and that the resulting transconjugants belonged to Enterobacteria and Pseudomonas. These findings suggest that the introduction of the pDOD plasmid or its donor into soil and its subsequent transfer to indigenous bacteria is a feasible bioaugmentation approach for the bioremediation of DDT-contaminated soil.

2.3. Effect of pDOD donors

The degradation of p,p′-DDT in the soil microcosms inoculated with strains D-6, TG I, TZ, D-6 (pDOD-gfp), TG I (pDOD-gfp), and TZ (pDOD-gfp) is shown in Fig. 4. At 120 days after inoculation, approximately 62.4%, 69.5%, and 65.3% of the added p,p′-DDT had been degraded by D-6 (pDOD-gfp), TG I (pDOD-gfp), and TZ (pDOD-gfp), respectively, which was much higher than the degradation observed in the soil inoculated with TG I (9.6%) or TZ (9.2%) without the pDOD plasmid. As shown in Table S2, the number of DDT degraders in the TG I (pDOD-gfp)-inoculated soil dramatically increased, from 2.0 × 10^5 CFU/g dry soil on day 0 to 35.5 × 10^7 CFU/g dry soil on day 60, and then remained at a high level above that observed in the other pDOD donor-inoculated soils. With increasing treatment time, the GFP fluorescence intensity in the soil bacteria in the TG I (pDOD-gfp)-inoculated soil was higher than that in the other pDOD donor-inoculated soils (Fig. S3). Throughout the experiment, no DDT degraders or fluorescence were observed in the soils inoculated with strains TG I and TZ without pDOD-gfp.

The transfer of a plasmid among soil bacteria is usually affected by the donor (Tsutsui et al., 2010). In this study, the performances of TG I (pDOD-gfp) and TZ (pDOD-gfp), as measured by DDT degradation, the MPN of DDT degraders and gfp expression, were found to be better than that of D-6 (pDOD-gfp) and D-6 alone. This difference may originate from the better adaptability of strains TG I and TZ to the soil used in the experiments compared with strain D-6, which was isolated elsewhere (Ueno et al., 2007; Brennan et al., 2013).

2.4. Environmental factors affecting plasmid transfer

Plasmid transfer can occur in the natural environment. However, environmental factors can influence the frequency of plasmid transfer. In terrestrial environments, the soil
temperature, soil moisture content and soil type have all been identified as factors influencing the frequency of plasmid transfer (Richaume et al., 1989, 1992). In this study, the effects of soil temperature, soil moisture and soil type on the MPN of degraders, the overall DDT degradation, and the gfp expression resulting from the transfer of the pDOD plasmid in soil were investigated with TG I (pDOD-gfp) as the plasmid donor.

The effect of temperature on the degradation of \( p,p' \)-DDT in TG I (pDOD-gfp)-inoculated soil is shown in Fig. 5A. After 120 days, approximately 63.2%, 70.0%, and 61.6% of the \( p,p' \)-DDT was degraded at 20, 30, and 40 °C, respectively. Thus, the degradation of \( p,p' \)-DDT was faster at 30 °C than at 20 or 40 °C. The number of degraders in the TG I (pDOD-gfp)-inoculated soil at 30 °C was also larger than that at 20 or 40 °C (Table S3). The most effective expression of gfp in the soil bacteria was observed at 30 °C rather than at 20 or 40 °C (Fig. S4). It is likely that the higher degradation of \( p,p' \)-DDT in the TG I (pDOD-gfp)-inoculated soil at 30 °C was the result of a more effective transfer of pDOD under this condition and a consequently higher MPN of degraders. In agreement with our results, the maximum transfer efficiencies of plasmids RP4 and pDOC were observed at approximately 30 °C in previous studies (Lafuente et al., 1996; Inoue et al., 2005; Zhang et al., 2012). Our results indicated that DDT degradation could be effectively enhanced by pDOD transfer in soil at all the tested temperatures; however, the optimal temperature was approximately 30 °C. It has been shown that a temperature range of 25–30 °C is optimal for the growth and activity of soil bacteria. The maximal performance observed at 30 °C might originate from the optimal growth and activity of the donor and recipients.

The influence of moisture on the degradation of \( p,p' \)-DDT in the TG I (pDOD-gfp)-inoculated soil is shown in Fig. 5B, where 60.7%, 65.3%, and 54.4% of the introduced \( p,p' \)-DDT were degraded at moistures of 40%, 60%, and 80% WHC, respectively. The maximal performance observed at 30 °C might originate from the optimal growth and activity of the donor and recipients.

The influence of moisture on the degradation of \( p,p' \)-DDT in the TG I (pDOD-gfp)-inoculated soil is shown in Fig. 5B, where 60.7%, 65.3%, and 54.4% of the introduced \( p,p' \)-DDT were degraded at moistures of 40%, 60%, and 80% WHC, respectively. The maximal performance observed at 30 °C might originate from the optimal growth and activity of the donor and recipients.

Fig. 5  Degradation of DDT in TG I (pDOD-gfp)-inoculated soil at different temperatures (A), at different soil moisture contents (B), and in different types of soils (C). TG I: Escherichia coli TG I; pDOD-gfp: gfp-tagged plasmid for the degradation of DDT.
The most profound decrease of \( p,p' \)-DDT in soil was observed at a moisture content of 60% WHC. Similarly, the largest number of degraders (Table S4) and the strongest expression of \( gfp \) (Fig. S5) were also detected at a moisture content of 60% WHC. These observations imply that a moisture level of 60% WHC is better suited than 40% or 80% WHC for the transfer of pDOD in soil. The soil moisture may alter the efficiency of plasmid transfer in soil by affecting the contact between the donor and the acceptor. It has been reported that soil with a moisture content of 60% WHC is favorable for plasmid transfer due to the optimal growth and activity of soil bacteria at this moisture level (Karpouzas and Walker, 2000; Miller et al., 2004; Aminov, 2011).

The impact of soil type on DDT degradation was smaller than the impacts of soil temperature or moisture. As shown in Fig. 5C, approximately 65.0%, 63.5%, 61.6%, and 60.1% of the added \( p,p' \)-DDT was degraded in the TG I (pDOD-\( gfp \))-inoculated soils HZ, XS, JX, and JH, respectively, after incubation for 120 days. However, the variations in the number of degraders with time and soil type revealed a complex dynamic process rather than a consistent trendline (Table S5). In light of the variation in the number of degraders throughout the experiment, soils HZ and JX were more amenable to the transfer of pDOD than soils XS and JH. This was verified by the GFP fluorescent intensity of the soils shown in Fig. S6. Soil pH is an important factor governing plasmid transfer in soil. It has previously been recognized that plasmid transfer occurs effectively in a neutral environment with a pH value of approximately 7.0 (Van Elsas et al., 2000; Zhang et al., 2012). The good performances of soils HZ and JX, as evidenced by their production of degraders and \( gfp \) expression, might be due to their pH values of near 7.0 (Table S1).

2.5. Application of plasmid transfer in DDT-contaminated field soil

The plasmid-mediated bioaugmentation method for enhancing the degradation of residual DDT in contaminated soil was performed in two adjacent fields located in Cixi, Zhejiang, China. The concentrations of residual DDT in the fields were determined prior to the experiments to be (0.60 ± 0.20) and (0.80 ± 0.08) mg/kg, respectively. Considering the fact that residual pesticides at low concentrations are degraded more slowly than the same chemicals at high concentrations, the contaminated fields containing DDT at (0.60 ± 0.20) and (0.80 ± 0.08) mg/kg were used as the treatment and the control, respectively. The dissipation patterns of DDT over time in the TG I (pDOD-\( gfp \))-treated field and the control field are shown in Fig. 6. Approximately 50.7% of the initial DDT was degraded in the TG I (pDOD-\( gfp \))-inoculated field 210 days after treatment, which was significantly greater than the amount degraded (16.2%) in the control soil without TG I (pDOD-\( gfp \)). In the soil that received TG I (pDOD-\( gfp \)), the MPN of DDT degraders increased successively from 2.00 × 10⁶ CFU/g dry soil on day 15 to 4.00 × 10⁷ CFU/g dry soil on day 210 (Table 2), and the corresponding GFP fluorescence intensity also increased gradually with treatment time (Fig. 7). The results indicated that the pDOD plasmid could effectively transfer among indigenous bacteria in the field and thus accelerate the degradation of DDT. This finding is in accord with Inoue et al. (2005), who found that plasmid transfer successfully mediated the bioremediation of 2,4-D-contaminated soil using E. coli HB101 as the carrier of plasmid pJP4.

![Graph showing DDT degradation over time](image)

**Fig. 6** – Degradation of DDT in field soil inoculated with TG I (pDOD-\( gfp \)). pDOD-\( gfp \): \( gfp \)-tagged plasmid for the degradation of DDT.

### 3. Conclusions

The results obtained in this study indicate that a desirable DDT-degrading property in soil could be acquired by the transfer of pDOD and its subsequent expression in indigenous bacterial hosts. The introduction of pDOD or its donor strain can be an effective bioaugmentation approach for the degradation of DDT in contaminated soils where indigenous bacterial communities lack the necessary metabolic potential. However, the plasmid transfer between soil bacteria is governed by many biotic and abiotic factors. More detailed studies of the influence of biological and environmental factors are necessary to improve our understanding and utilization of plasmid-mediated bioaugmentation.

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Control</th>
<th>E. coli TG I (pDOD-( gfp ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>ND</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>30</td>
<td>ND</td>
<td>0.028 ± 0.015</td>
</tr>
<tr>
<td>60</td>
<td>ND</td>
<td>1.03 ± 0.12</td>
</tr>
<tr>
<td>90</td>
<td>ND</td>
<td>14.3 ± 2.57</td>
</tr>
<tr>
<td>120</td>
<td>ND</td>
<td>9.5 ± 2.0</td>
</tr>
<tr>
<td>150</td>
<td>ND</td>
<td>7.83 ± 2.89</td>
</tr>
<tr>
<td>180</td>
<td>ND</td>
<td>5.83 ± 1.15</td>
</tr>
<tr>
<td>210</td>
<td>ND</td>
<td>4.00 ± 1.32</td>
</tr>
</tbody>
</table>

ND: not detectable.
Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2014.05.045.

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