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ORIGINAL PAPER

Simultaneous biodegradation of methyl parathion and carbofuran by a genetically engineered microorganism constructed by mini-Tn5 transposon

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Abstract A genetically engineered microorganism (GEM) capable of simultaneous degrading methyl parathion (MP) and carbofuran was successfully constructed by random insertion of a methyl parathion hydrolase gene *(mpd)* into the chromosome of a carbofuran degrading *Sphingomonas* sp. CDS-1 with the mini-transposon system. The GEM constructed was relatively stable and cell viability and original degrading characteristic was not affected compared with the original recipient CDS-1. The effects of temperature, initial pH value, inoculum size and alternative carbon source on the biodegradation of MP and carbofuran were investigated. GEM cells could degrade MP and carbofuran efficiently in a relatively broad range of temperatures from 20 to 30°C, initial pH values from 6.0 to 9.0, and with all initial inoculation cell densities (10^5–10^7 CFU ml^{-1}), even if alternative glucose existed. The optimal temperature and initial pH value for GEM cells to simultaneously degrade MP and carbofuran was at 30°C and at pH 7.0. The removal of MP and carbofuran by GEM cells in sterile and non-sterile soil were also studied. In both soil samples, 50 mg kg^{-1} MP and 25 mg kg^{-1} carbofuran could be degraded to an undetectable level within 25 days even if there were indigenous microbial competition and carbon sources effect. In sterile soil, the biodegradation rates of MP and carbofuran were faster, and the decline of the inoculated GEM cells was slower compared with that in non-sterile soil. The GEM constructed in this study was potential useful for pesticides bioremediation in natural environment.

Keywords Biodegradation · Carbofuran · Genetically engineered microorganism · Methyl parathion · Mini-transposon

Abbreviations

Carbofuran	2,3-dihydro-2, 2-dimethylbenzofuran-7-yl methylcarbamate
GEM	Genetically Engineered microorganism
MP	*O*, *O*-dimethyl *O*-4-nitrophenyl phosphorothioate

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mpd methyl parathion hydrolase gene
PNP *p*-nitrophenol

Introduction

Chemical pesticides are frequently applied in modern agricultural system to ensure good harvests. However, the extensive use of chemical pesticides may easily lead to widespread environmental pollution, resulting in serious damage to non-target species. Methyl parathion (*O*,*O*-dimethyl *O*-4-nitrophenyl phosphorothioate, MP) and carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl methylcarbamate) (chemical structures shown in Fig. 1) are both broad-spectrum pesticides widely used in agricultural practice throughout the world (Bondarenko et al. 2004). Toxicity of these pesticides has been correlated with their inhibitory effect on acetylcholinesterase activity at central cholinergic and at neuromuscular junctions (Gupta 1994; Bretaud et al. 2000).

The control of pesticides pollutants is of great importance because they are toxic and recalcitrant. Several decontamination techniques are available for the removal of these contaminants, although not all are efficient enough to destroy the contaminants. Microorganisms play important roles in the detoxification of xenobiotic compounds, and can potentially degrade these contaminants to innocuous products (mainly CO_2 and H_2O). The use of microorganisms for bioremediation of pesticides-contaminated sites may prove to be a viable alternative to physical and chemical clean-up methods, because a variety of microorganisms are known to utilize pesticides as their sole carbon or energy source.

Some microorganisms capable of degrading organophosphorus pesticides including MP have been isolated (Serdar et al. 1989; Cui et al. 2001; Liu et al.1999), and an organophosphorus hydrolyse gene (*opd*) has been characterized (Mulbry et al. 1986; Serdar et al. 1989). The *opd* gene has been cloned into *Escherichia coli* (Serdar et al. 1989), *Streptomyces* (Steiert et al. 1989), and soil fungus (Dave et al. 1994). Recombinant OPHs have been demonstrated to be equally effective in hydrolyzing various organophosphorus pesticides and nerve agents. Another methyl parathion hydrolase (*mpd*) gene, responsible for hydrolyzing MP to *p*-nitrophenol (PNP) and dimethyl phosphorothinate at high efficiency was cloned and has been expressed in *E. coli* and *Bacillus subtilis* WB800 in our lab (Cui et al. 2001; Liu et al. 2003; Zhang et al. 2005a, b).

Several microorganisms capable of transforming carbofuran have been also isolated (Karns et al. 1986; Chaudhry and Ali 1988; Ramanand et al. 1988; Feng et al. 1997; Bano and Musarra 2004; Kim et al. 2004). The pathway of these bacteria used to degrade carbofuran has been studied (Ramanand et al. 1988; Karns et al. 1986; Feng et al. 1997; Chaudhry and Ali 1988). Chaudhry et al. (2002) reported that carbofuran could be transformed to 7-phenol (2,3-dihydro-2,2-dimethyl-7-hydroxy benzofuran) and 4-hydroxycarbofuran (2, 3-dihydro-2, 2-dimethyl-4-hydroxybenzofuran-7-yl methylcarbamate) by *Pseudomonas* sp. 50432. Kim et al. (2004) reported that a *Sphingomonas* sp. strain SB5 could hydrolyze carbofuran to 2-hydroxy-3-(3-methylpropan-2-ol) phenol at the furanyl ring. In our lab, a carbofuran degrading strain *Sphingomonas* sp. CDS-1, which could use carbofuran as the sole carbon and energy source was isolated and characterized (Wu et al. 2004). Carbofuran-phenol was identified as the initial metabolite and 2, 4-bis-tertiary butyl-quinone was also detected in the process of carbofuran degradation by GC/MS analysis (Wu et al. 2004).

Because of the various kinds of pesticide-residues present in environment, multifunctional genetically engineered microorganisms (GEMs) are needed to clean up these pollutants (Carlos and Itziar 1999; Chen et al. 1999). However, GEMs capable of simultaneous degrading MP and carbofuran have not been studied extensively. In the present study, the *mpd* gene was randomly inserted into the chromosome of the carbofuran

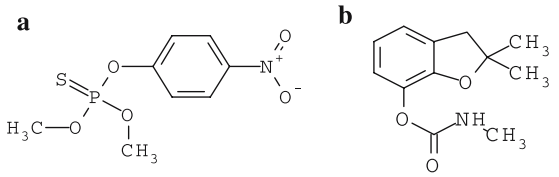

Fig. 1 Chemical structures of MP (**a**) and carbofuran (**b**)

degrading *Sphingomonas* sp. CDS-1 to construct a GEM by a mini-Tn transposon. To gain a better understanding of this newly constructed GEM and to explore its potential for pesticide detoxification, several factors influencing the pesticides degradation, and the simultaneous biodegradations of pesticides in sterile and non-sterile soil by GEM cells were also investigated.

Materials and methods

Chemicals and enzymes

Carbofuran (98% purity) and MP (50 and 98% purity) were purchased from Paoligehua (Taichang) Co. Ltd. and Zhenjiang pesticide factory (Zhenjiang), respectively. All other chemicals used were analytical grade and purchased from Chuangrui Co. Ltd., China. Restriction enzymes, T4 DNA ligase and Calf Intestinal phosphatase were purchased from TaKaRa Biotechnology (Dalian) Co. Ltd. Specific primers used for PCR amplification were synthesized by Bioasia Co. Ltd. (Shanghai). All DNA manipulations were performed according to standard procedures (Sambrook et al. 1989).

Strains and growth conditions

Carbofuran degrading strain *Sphingomonas* sp. CDS-1 [wild type, resistant to streptomycin (Str) and ampicillin (Amp)] was cultured in 1/3 diluted Luria-Bertani (1/3 LB) medium (3.3 g of tryptone, 1.7 g yeast extract and 3.3 g NaCl per liter, pH 7.0) or minimal salt medium (MSM) (2.0 g Na_2HPO_4, 0.75 g KH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 1.0 g NH_4Cl per liter, pH 7.0) supplemented with 100 μg ml^{-1} carbofuran (100 μg ml^{-1} MP was added if needed) at 30°C. *E. coli* strains were cultured in LB medium at 37°C. For solid media, 20.0 g of agar per liter was added. Antibiotics were added if required at the following concentrations: streptomycin (Str), 100 μg ml^{-1}; ampicillin (Amp), 50 μg ml^{-1}; kanamycin (Km), 25 μg ml^{-1}, chloramphenicol (Cm), 20 μg ml^{-1}. *E. coli* DH5$\alpha_{\lambda pir}$ [*RP4-2-tet:Mu-kan::Tn7 integrant leu–63::IS10 recA1 creC510 hsdR17 endA1 zbf-5 uidA(*ΔMluI): pir^+ *thi*] expressing R6K π replicase was used as the host cell for the R6K-based suicide delivery plasmid pUT-Km (Victor et al. 1990). pUT-Km provides the IS*5*O_R transposase *tnp* gene in *cis* but external to the mobile element and whose conjugal transfer to recipients is mediated by RP4 mobilization functions. Mini-Tn5 transposon located on the pUT-Km consists of gene specifying resistance to Km as selection marker and a unique *Not*I cloning site flanked by 19-base-pair I and O Tn5 ends. *E. coli* HB101 (pRK600) was used as the helper strain in triparental mating. The plasmid pRK600 encodes the gene resistant to Cm and provides the *tra* function for mobilization of the pUT-Km plasmids.

Construction of pUT-Km-mpd

The functional *mpd* gene fragment with its promoter was amplified with the primer pair pNot1 (5′-TA*GCGGCC*GCTCCGTCCAATCTCC-3′)/pNot2 (5′-TA*GCGGCC*GCTATCACTTGGGGTTG-3′) with pDT3 (Liu et al. 2003) as the template. *Not*I site (italic) and additional stop codon (TGA) were introduced with primers. PCR amplification was performed in a total volume of 25 μl containing 1 μl of template DNA, 5 pmol of each primer, 5 pmol of dNTPs (Genebase), 2.5 μl of 10× reaction buffer, 3 μl of 25 mM $MgCl_2$ and 1 U of Taq DNA polymerase (Genebase). Thermal cycling was performed on a Peltier Thermal cycler (PTC-200, MJ Research) with an initial denaturation at 95°C for 2 min followed by 30 cycles consisting of denaturation at 96°C for 0.5 min, annealing at 60°C for 0.5 min, and extension at 72°C for 1.5 min, and a single final extension at 72°C for 10 min. The PCR products were purified and digested with *Not*I and ligated to the unique *Not*I cloning site of the pUT-Km. The ligation products were transformed into the competent *E. coli* DH5$\alpha_{\lambda pir}$ cells, and the transformants forming yellow transparent halos of PNP around the colonies on LB agar plates supplemented with Amp (100 μg ml^{-1}), Km (25 μg ml^{-1}) and MP (100 μg ml^{-1}) were selected. The constructed vector for random insertion of *mpd* gene into the chromosome of gram-negative bacteria was designated as pUT-Km-mpd, and shown in Fig. 2.

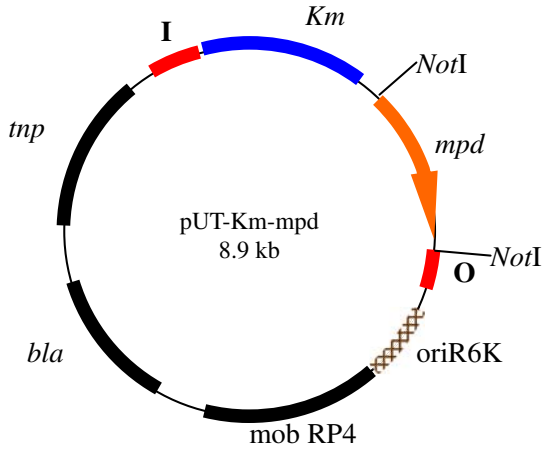

Fig. 2 Map of the plasmid pUT-Km-mpd

Random insertion of *mpd* gene into the chromosome of *Sphingomonas* sp. CDS-1

Donor strains *E. coli* DH5$\alpha_{\lambda pir}$ harboring the plasmid pUT-Km-mpd were mixed with Str^r recipient strains *Sphingomonas* sp. CDS-1 and the help strains *E. coli* HB101 (pRK600) at a ratio of 2:4:1 and then filtered through a 0.45 μm cellulose nitrate membrane filter. The filter was then placed on the surface of 1/3 LB agar plate and further incubated for 24 h. The cells on the filter surface were then suspended in sterile H_2O, and the suspension was spread onto 1/3 LB agar plates containing 100 μg ml^{-1} Str and 25 μg ml^{-1} Km. The exconjugants confirmed able to hydrolyze MP were selected for further study.

Pesticides biodegradation tests

Unless stated otherwise, the pesticides biodegradation tests were carried out as below: GEM cells cultured in 1/3 LB medium were harvested during log phase and centrifuged at 7,800×*g* for 5 min, the pellet washed twice with MSM medium was used as inoculum. One milliliter of the washed GEM cells was inoculated to 100 ml of MSM medium (pH 7.0) containing 100 μg ml^{-1} carbofuran and 100 μg ml^{-1} MP as the source of carbon and energy. Cultures were maintained in 250 ml bottles at 30°C on a shaker operated at 160 rpm. Negative controls were inoculated with sterilized GEM cells (autoclaved at 121°C for 15 min) under the same conditions. At different time periods, sample aliquots of 2 ml were removed for analysis.

To investigate the effect of temperature on pesticides biodegradation at pH 7.0, the cultures were shaken at 25, 30, 35 and 40°C, respectively. To investigate the effect of initial pH value of the medium on pesticides biodegradation at 30°C, the pH values of the media were adjusted to 4.0, 6.0, 7.0 and 9.0, respectively, by changing the potassium-phosphate buffer system. To investigate the effect of inoculum size on pesticides biodegradation at pH 7.0 and 30°C, 0.1, 1 and 10 ml of the GEM cells were inoculated to mediums, respectively. To investigate the effect of alternative carbon source on pesticides biodegradation at pH 7.0 and 30°C, 100 μg ml^{-1}, 1 mg ml^{-1} and 10 mg ml^{-1} glucose were additionally added to mediums, respectively.

Pesticides biodegradation by GEM cells in soil

Soil samples were collected from agricultural fields in Jiangsu province and some properties of the soil samples are listed in Table 1. Soil sample sets (500 g) were air-dried, sieved to 2 mm, homogenized. Carbofuran and MP were added to soil at concentrations of 25 and 50 mg a.i. kg^{-1} dry soil, respectively. The treated soil was inoculated with the GEM cells and the control soil with the sterilized GEM cells (autoclaved at 121°C for 15 min). All the treatments were replicated three times. Every soil sample set was put into separate pot and placed at the room temperature. Distilled water was added to adjust the moisture contents to 40% of soil maximum holding water capacity. To prevent the photodegradation of the pesticides in soil, the pots were kept covered with a black sheet. During incubation, 25 g sub-samples were

Table 1 Selected properties of the soil tested

pH	7.65
Organic carbon (g kg^{-1})	13.56
Water holding capacity (%)	32.40
Total N (g kg^{-1})	0.79
Available N (mg kg^{-1})	75.62
Total P (g kg^{-1})	0.19
Available P (mg kg^{-1})	4.02
Total K (g kg^{-1})	17.54
Available K (mg kg^{-1})	102.42

collected at periodic intervals from the replicated pots of each treatment and were analyzed immediately. The biodegradations of pesticides by GEM cells in sterile soil were carried out with the soil samples sterilized at 121°C for 30 min for twice. Sterility of the autoclaved soil was carefully checked by the plating method, and no microorganism growth was found.

Analytical methods

The cell density was monitored spectrophotometrically by measuring the absorbance at 600 nm by using a SHIMADZU UV–Vis Recording spectrophotometer. MP in soil was extracted with acetone:water (80:20, v/v), and the organic layer was filtered through anhydrous sodium sulfate. The extract was then evaporated with nitrogen to a final volume of 1 ml. MP and PNP were determined by the method described previously (Zhang et al. 2005a, b). Carbofuran in aqueous phase or in soil samples was extracted with acetone for twice, and the total extract collected was filtered through anhydrous sodium sulfate. The extract was evaporated to a final volume of 1 ml under a gentle stream of nitrogen. Carbofuran was measured by using the UV–Vis Recording spectrophotometer at A_{282} nm compared with a standard curve. The GEM cells in soil samples at each time were estimated by standard dilution plating techniques on 1/3 LB agar plates containing 100 μg ml^{-1} Str, 50 μg ml^{-1} Amp, 25 μg ml^{-1} Km and 100 μg ml^{-1} MP, and colony-forming unit (CFU) with yellow halo enumerated following 72 h incubation at 30°C.

Results and discussions

Construction of GEM

The *mpd* gene was randomly inserted into the chromosome of *Sphingomonas* sp. CDS-1 by triparental mating as described in Materials and methods. Since pUT-Km-mpd carrying the $R_{R6K\gamma}$ DNA replicate origin is not able to replicate in *Sphingomonas* sp. CDS-1 (devoid of π replication protein), only transposition events of mini Tn5 give rise to Km resistant exconjugants. This procedure allows isolation of exconjugants with *mpd* gene inserted into chromosome. The *mpd* gene inserted into chromosome of CDS-1 was verified by performing PCR. Genomic DNA of the exconjugants were extracted and amplified with the primer pair pNotI1/pNotI2. Specific 1.3-kb long products could be amplified from all exconjugants genomic DNA indicating that *mpd* gene was located in chromosome (data not shown). The growth and carbofuran degrading abilities of ten randomly selected exconjugants were investigated. Results demonstrated that the growth curves and carbofuran degrading characteristics of these exconjugants were similar to that of the wild-type CDS-1, showing that random insertion of *mpd* gene in these strains had not affected cell viability and original degrading characteristics. The MP degrading abilities of these selected exconjugants were almost the same, indicting that in most cases only one *mpd* gene was inserted into chromosome. One exconjugant was selected as the GEM for further study, and its scanning electronic microscope and growth curve in MSM medium supplemented with 100 μg ml^{-1} carbofuran compared with that of the wild-type CDS-1 are shown in Figs. 3 and 4.

In order to check the stability of the GEM, the GEM cells were grown in 1/3 LB medium without antibiotics at 30°C and then transferred to the next culture under the same conditions. The procedure was repeated a sixth time. Then, 100 μl of a 10^{-8} dilution was plated on 1/3 LB agar plate with 100 μg ml^{-1} MP and without any antibiotic. After 72 h incubation, colonies formed on plates were examined for the yellow transparent halos of PNP around colonies. We examined over 200 colonies formed by GEM cells and found that all colonies had yellow halos. The stability of *mpd*

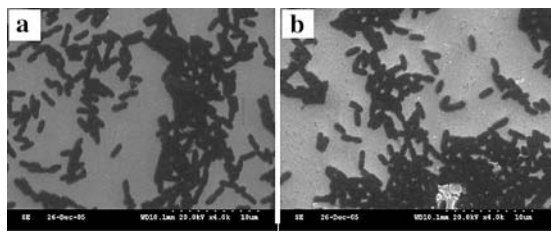

Fig. 3 Micrographs of the *Sphingomonas* sp. CDS-1 (**a**) and GEM (**b**) under scanning electronic microscope (×4,000)

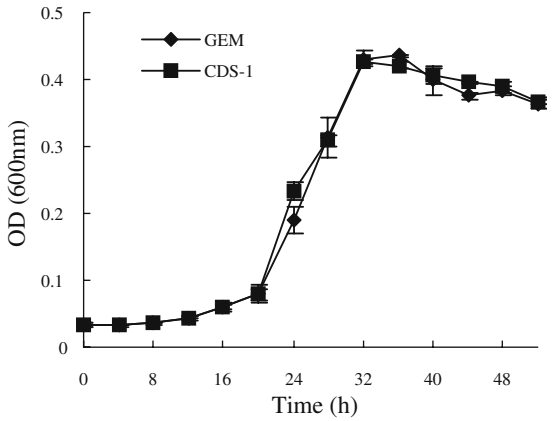

Fig. 4 Growth curves of GEM and *Sphingomonas* sp. CDS-1 in MSM medium containing 100 μg ml^{-1} carbofuran. The data are represented as the means±standard deviation for triplicate incubations. When the *error bar* is not visible it is within the data point

gene in chromosome was also certified by PCR method. We found that the specific 1.3-kb *mpd* gene fragment could be amplified from all 50 randomly selected colonies with the primer pair pNot1/pNot2. These results indicated that no loss of *mpd* gene occurred after growth at least 100 generations and showing that the GEM constructed was relatively stable.

Effect of temperature on pesticides biodegradation

It was reported that temperature might have the large role in degrading organic pollutants (Margesin and Schinner 1997). The biodegradations of MP and carbofuran by GEM cells at different temperatures were investigated (Fig. 5). With the temperature increased from 20 to 35°C, the degrading rates of MP were also increased and the maximum degrading rate was at 35°C. Under all temperatures tested, 100 μg ml^{-1} MP could be removed to an undetectable level (< 0.02 μg ml^{-1}) within 18 h. For carbofuran, the suitable temperature for GEM cells to degrade was from 20 to 30°C and the optimal degrading temperature was 30°C. At 35°C, growth of the GEM cells was slow, and only about 45% of carbofuran was removed after 32 h and carbofuran was degraded to an undetectable level (< 0.1 μg ml^{-1}) in 40 h. Even though the degradation of carbofuran at 35°C was slight slow, there was a relatively broad range of temperatures for GEM cells to simultaneously degrade these two pesticides.

Effect of initial pH value of the medium on pesticides biodegradation

Environmental factors such as pH may affect the growth of microorganisms and their degradational abilities. In the present study, four initial pH values of the media from 4.0 to 9.0 were investigated (Fig. 6). GEM cells could rapidly degrade MP and carbofuran in a range of initial pH values from 6 to 9. At initial pH value of 6.0, 7.0 and 9.0, pesticide degradation rate was nearly the same. The optimal initial pH for MP and carbofuran degradation was at 7.0. It was possible

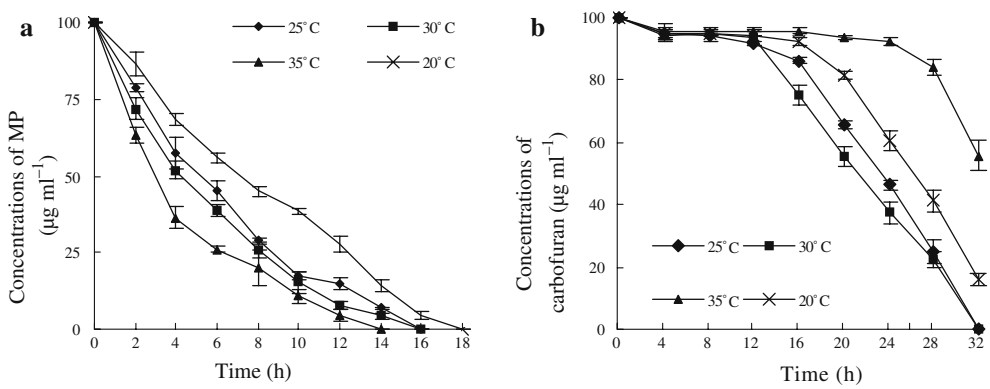

Fig. 5 MP (**a**) and carbofuran (**b**) biodegradation by GEM cells at different temperatures. Negative controls exhibited no significant change in pesticides concentrations during incubation. The data are represented as the means ± standard deviation for triplicate incubations. When the *error bar* is not visible it is within the data point

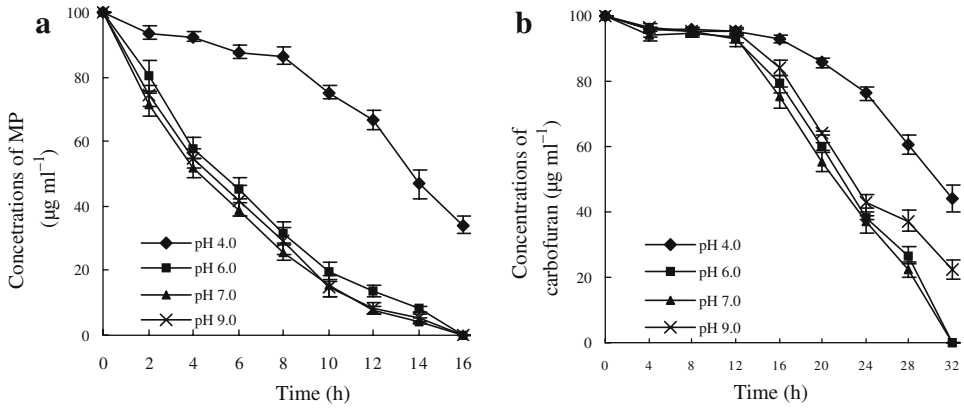

Fig. 6 MP (**a**) and carbofuran (**b**) biodegradation by GEM cells at different initial pH values. Negative controls exhibited no significant change in pesticides concentrations during incubation. The data are represented as the means ± standard deviation for triplicate incubations. When the *error bar* is not visible it is within the data point

that GEM cells grown well at pH 7.0 and the enzymes for MP and carbofuran degradation had their optimum enzymatic activities. The MP and carbofuran degradations at pH 4.0 were little slower, and about 66% of MP and 56% of carbofuran were degraded in 16 h and 32 h, respectively.

Effect of inoculum size on pesticides biodegradation

Methyl parathion and carbofuran were degraded by GEM cells during all the initial cell densities tested, and pesticides degradations increased linearly with the increase of inoculum sizes. In cultures inoculated with the highest cell density (about $2.51 \pm 0.53 \times 10^7$ CFU ml^{-1}) the MP and carbofuran concentrations were degraded rapidly after 0.5 h and 10 h, and degraded to an undetectable level within 10 h and 26 h, respectively. In cultures receiving lower inoculum densities ($2.5 \pm 0.53 \times 10^5$ CFU ml^{-1}), the contents of MP and carbofuran began to decrease after 4 h and 20 h, and degradations were completed after 18 h and 40 h, respectively.

Effect of alternative carbon source on pesticides biodegradation

It has been suggested that alternative nutrient availability could affect the bacterial growth and substrates degradation (Roszak and Colwell 1987; Watanabe et al. 1998). In this work, we found that low concentrations of glucose (100 μg ml^{-1} and 1 mg ml^{-1}) existed had almost no effect on the degradation of both pesticides. However, supplementary of 10 mg ml^{-1} glucose delayed the degradation of carbofuran, even though the GEM cells grew better. The time for complete degradation of carbofuran was delayed for 3 h, from 32 h to 35 h. The phenomenon was most likely due to the existence of high concentrations of more available alternative sources of carbon and energy. MP degradation was not affected by glucose because MP hydrolase is expressed constitutively and hydrolyzes MP to PNP constantly.

MP and carbofuran degradation versus GEM cells growth

When 1 ml of inoculation of GEM cells were transferred to the MSM medium containing pesticides as carbon and energy source, cell growth did not occur for the first 16 h. MP was completely degraded and PNP (the product of MP hydrolysis) was accumulated during this lag phase, even though carbofuran remained nearly undegraded. After the lag phase, there was a rapid exponential growth phase. The turbidity of the culture (OD_{600}) increased to 0.31 at 32 h and carbofuran was degraded to an undetectable level over this period (Fig. 7).

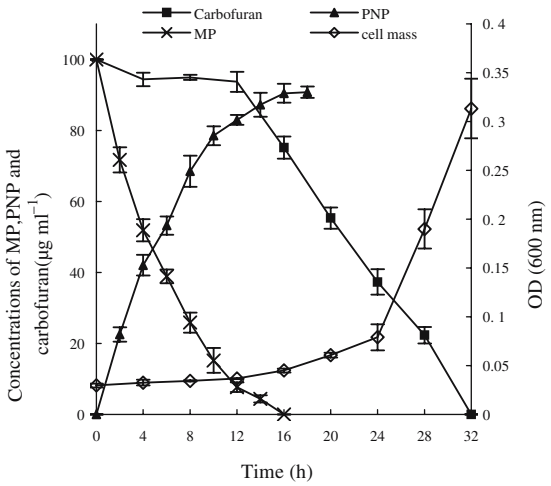

Fig. 7 Simultaneous biodegradation of MP, carbofuran and accumulation of PNP versus GEM cells growth in MSM medium supplemented with 100 μg ml^{-1} carbofuran and 100 μg ml^{-1} MP at 30°C. The data are represented as the means±standard deviation for triplicate incubations. When the *error bar* is not visible it is within the data point

Pesticides biodegradation in soil

In order to determine whether GEM cells degraded the target MP and carbofuran in soil and to examine the growth of the GEM cells when in competition with indigenous bacteria and in the presence of alternative carbon sources, sterile and non-sterile soil was used, and inoculated with GEM cells. Bacterial counts on Str, Km and MP plates show that the initial inoculation of GEM cells in both soil was 5.3 ±0.79 × 10^7 CFU g^{-1} dry soil. Nearly no degradation of carbofuran took place in both control soil samples. However, about 30% of MP was degraded in 25 days in non-sterile soil most likely due to the indigenous microbial transformation.

In our study, the rate of MP dissipation was higher than that of carbofuran in both soil samples, as the total loss of MP occurred in no more than 15 days compared with that of carbofuran in 25 days (Fig. 8). In sterile soil, the degradation rates of MP and carbofuran by GEM cells were faster, and the numbers of GEM cells decreased slower than those in non-sterile soil, suggesting that there was a competition between the indigenous population and the inoculated GEM strains. The effectiveness of bioremediation depends both on the degrading microorganisms and the contaminants bioavailability (Cort and Bielefeldt 2000; Das and Mukherjee 2000). Bioavailability of contaminants is affected mostly by many physical, chemical and structural properties of both the contaminants and the soil matrix (Loh and Wang 1998). In the present study, two pesticides could be completely degraded within 25 days in both soil samples, indicating that the GEM constructed was potential useful for pesticides bioremediation in natural environment even if there were indigenous microbial competition and carbon sources effect.

The GEM strains could not be detected after 20 and 15 days in sterile and non-sterile soil,

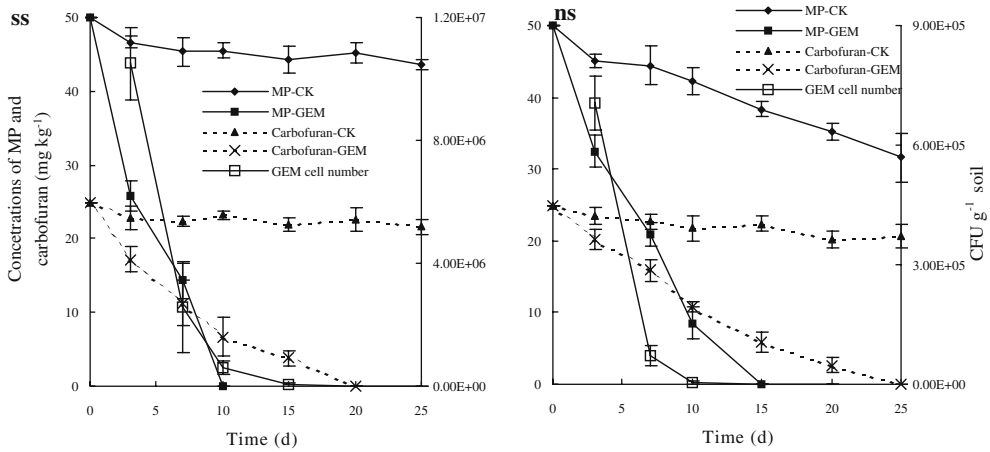

Fig. 8 Biodegradation of MP (50 mg kg^{-1}) and carbofuran (25 mg kg^{-1}) added to sterile soil (*ss*) and non-sterile soil (*ns*) by GEM and sterilized GEM cells. The data are represented as the means ± standard deviation for triplicate experiments. When the *error bar* is not visible it is within the data point

respectively, mostly due to the limitations of the plate counting technology. However, declines of GEM strains in both soil samples were observed, indicating that the GEM would not be the predominant population in soil. These results showed that the GEM was of importance in natural environmental bioremediation because of its low risk of environmental release.

Conclusions

In the present study, a GEM capable of simultaneous degrading MP and carbofuran, was successfully constructed by a mini-transposon system. The GEM constructed was relatively stable and would not lose MP hydrolysis abilities after at least 100 generations cultured in none selective pressure. The changes of temperatures, initial pH values of mediums, inoculum sizes and alternative glucoses affected the rates of pesticides biodegradations. However, the GEM was able to simultaneously degrade MP and carbofuran over a wide range of temperatures, pHs and initial inoculation cell densities, even if alternative carbon source existed. In both sterile and non-sterile soil samples, 50 mg kg^{-1} MP and 25 mg kg^{-1} carbofuran could be degraded to an undetectable level by GEM cells within 25 days even if there were indigenous microbial competition and carbon sources effect.

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