Metabolic Engineering of *Pseudomonas putida* KT2440 for Complete Mineralization of Methyl Parathion and γ-Hexachlorocyclohexane

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**ABSTRACT:** Agricultural soils are often cocontaminated with multiple pesticides. Unfortunately, microorganisms isolated from natural environments do not possess the ability to simultaneously degrade different classes of pesticides. Currently, we can use the approaches of synthetic biology to create a strain endowed with various catabolic pathways that do not exist in a natural microorganism. Here, we describe the metabolic engineering of a biosafety *Pseudomonas putida* strain KT2440 for complete mineralization of methyl parathion (MP) and γ-hexachlorocyclohexane (γ-HCH) by functional assembly of the MP and γ-HCH mineralization pathways. The engineered strain was genetically stable, and no growth inhibition was observed. Such a strain not only would reduce the toxicity of MP and γ-HCH but also would prevent the accumulation of potentially toxic intermediates in the environment. Furthermore, expression of *Vitreoscilla* hemoglobin improved the ability of the engineered strain to sequester O₂. The inoculation of the engineered strain to soils treated with MP and γ-HCH resulted in a higher degradation rate than in noninoculated soils. Moreover, introduced GFP may be used to monitor the activity of the engineered strain during bioremediation. The engineered strain may be a promising candidate for *in situ* bioremediation of soil cocontaminated with MP and γ-HCH.

**KEYWORDS:** methyl parathion, γ-hexachlorocyclohexane, complete mineralization, *Pseudomonas putida* KT2440

Organophosphates (OP) such as methyl parathion (MP) are highly toxic to humans by virtue of their potent inhibition of acetylcholinesterase. Approximately 3 million poisonings and 300,000 human deaths occur per year owing to OP ingestion. Organochlorines (OC) such as γ-hexachlorocyclohexane (γ-HCH) are highly persistent in the environment and tend to accumulate in biological tissues. Because agricultural soils are usually cocontaminated with MP and γ-HCH and microorganisms capable of simultaneously degrading MP and γ-HCH have not been isolated from natural environments so far, genetically engineered microorganisms with the capability to simultaneously degrade MP and γ-HCH are useful in the remediation of the combined pollution caused by MP and γ-HCH. In our previous study, *Pseudomonas putida* KT2440 has been genetically engineered for simultaneous degradation of organophosphates and pyrethroids.

Microorganisms play the most important role in degradation of pesticides in soils. The complete mineralization pathways for MP and γ-HCH have been intensively studied in some model strains such as *Pseudomonas* sp. WBC-3 and *Sphingobium japonicum* UT26. Unfortunately, microorganisms capable of simultaneously mineralizing MP and γ-HCH have not yet been isolated from natural environments.

To avoid the accumulation of toxic degradation products, complete mineralization of synthetic pesticides is more desirable than detoxification, but also more difficult, requiring assembly of multistep pathways to convert the pesticide into an intermediate in a standard metabolic pathway. With the rapid development of metabolic engineering, the complete heterologous catabolic pathway rather than a single gene can be introduced into a host strain. For example, a toxic persistent pollutant, 1,2,3-trichloropropane (TCP), can be converted to harmless glycerol by functional assembly of a heterologous catabolic pathway composed of three enzymes from two different microorganisms in *Escherichia coli*. A mineralization pathway for a highly toxic organophosphorus pesticide, paraoxon, was functionally assembled in *P. putida* by introducing an organophosphorus hydrolase gene (*opd*) and a *pnp* operon encoding enzymes that transform *p*-nitrophenol into β-ketoadipate.

*P. putida* KT2440 is a soil-dwelling bacterium to be certified as a safety strain by the Recombinant DNA Advisory Committee and has been highlighted as an optimal chassis.
for implantation of synthetic genetic circuits. Moreover, a genome editing method based on the use of upp as a counter-selectable marker had been established in P. putida KT2440. In P. putida KT2440, the upp gene is nonessential for cell survival.

In this study, the complete mineralization pathways for MP and γ-HCH were functionally assembled in P. putida KT2440 by scarless integration of multiple degradation genes into the chromosome using upp as a counter-selectable marker. Pathways for complete mineralization of MP and γ-HCH assembled in P. putida KT2440 are shown in Figure 1. The resulting strain was capable of simultaneously mineralizing MP and γ-HCH. Moreover, vgb gene encoding Vitreoscilla hemoglobin (VHb) and gfp gene encoding enhanced green fluorescent protein (GFP) were introduced.

### RESULTS AND DISCUSSION

#### Construction of the Mineralization Pathways of MP and γ-HCH in P. putida KT2440

In most cases, the toxicity of one pollutant always can be potentiated by the presence of other pollutants. For example, coexposure to OP and OC insecticides can potentiate the toxicity of the individual compounds. Bioremediation has received increased attention as a simple, safe, and cost-effective technique to remediate pesticide-contaminated environments. To remediate the combined pollution caused by multiple pesticide residues, two alternative strategies, including the use of multiple degraders with distinct metabolic capabilities and assembly of diverse catabolic pathways from various degraders in a single microorganism, have been proposed so far.

To completely mineralize MP and γ-HCH, in this study, the MP and γ-HCH mineralization pathways comprising seven enzymes from three different microorganisms were assembled in P. putida KT2440 (Figure 1). All genes (including mpd, pnpA, pnpB, gfp, linA, linB, linC, linD and vgb) were integrated into the chromosome of P. putida KT2440 using a previously developed scarless gene replacement procedure with upp as a counter-selectable marker. Scheme of upp-based counter-selection system and assembly of gene expression elements are shown in the Supporting Information, Figure S1.

The successful construction of P. putida KTU-9 carrying nine exogenous genes was verified by PCR and DNA sequencing. The specific DNA fragments, which matched well with the size of nine exogenous genes, were obtained by PCR from the chromosomal DNA of P. putida KTU-9 (Figure 2) and the nucleotide sequences of the amplified DNA fragments were consistent with those of nine exogenous genes (Figure S2).

#### Confirmation of Transcription of the Inserted Genes in P. putida KTU-9

To detect the transcription of the inserted genes in P. putida KTU-9, PCR assays were performed with mRNA molecules extracted from the cells of P. putida KTU-9. After reverse transcription and PCR amplification, PCR products were detected by agarose gel electrophoresis. As expected, nine exogenous genes (mpd, pnpA, pnpB, gfp, linA, linB, linC, linD, and vgb) were amplified by PCR with the cDNA or genomic DNA as the template, however, they were

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**Figure 1.** Pathways for complete mineralization of methyl parathion and γ-hexachlorocyclohexane assembled in P. putida KT2440. Enzyme sources: MPH derived from Stenotrophomonas sp. YC-1, PnpAB derived from Pseudomonas putida DLL-E4, and LinABCD derived from Sphingobium japonicum UT26.

**Figure 2.** Detection of the introduced nine genes in P. putida KTU-9 by PCR and agarose gel electrophoresis. A specific DNA band for each gene that matched well with the size of each target gene was obtained by PCR from the chromosome using the gene-specific primers. Samples: lane M, DNA marker; lane 1, mpd; lane 2, pnpA; lane 3, pnpB; lane 4, gfp; lane 5, linA; lane 6, linB; lane 7, linC; lane 8, linD; lane 9, vgb.
genes were successfully transcribed to mRNA in *P. putida* KTU-9. Expression of all these introduced genes was under the control of a constitutive promoter J23119.

**Evaluating the Capability of *P. putida* KTU-9 for Complete Mineralization of MP and γ-HCH.** MP hydrolysis and γ-HCH dechlorination produce toxic and recalcitrant intermediates. Therefore, it is necessary to create engineered strains with the capability to completely eliminate these toxic degradation products. To verify whether *P. putida* KTU-9 was endowed with the ability to completely mineralize MP and γ-HCH, degradation experiments with *P. putida* KTU-9 were performed using an initial optical cell density (OD_{600}) of 0.05 in M9 minimal medium supplemented with MP, γ-HCH, PNP, 2,5-DCHQ, or HQ. At different time points, samples were removed for measuring the concentration of substrate by GC–MS. As shown in Figure 4, 100 mg/L MP and γ-HCH were completely degraded within 12 and 52 h, respectively, and 500 mg/L HQ, PNP, and 2,5-DCHQ were completely degraded within 16, 20, and 32 h, respectively. In contrast, when inoculated with *P. putida* KT2440, the concentration of MP, γ-HCH, PNP, and 2,5-DCHQ did not change in the media, but HQ reduction was observed because *P. putida* KT2440 has an intrinsic capability for HQ degradation. These results indicated that the observed substrate reduction was attributed to the occurrence of biodegradation.

In GC–MS analysis of the cultures grown on MP, γ-HCH, 2,5-DCHQ, PNP, or HQ, five characteristic peaks with a retention time (RT) of 9.905, 14.56, 9.43, 7.404, and 5.727 min, respectively, appeared in GC separation, and their MS spectra had a molecular ion peak at *m/z* = 262.9, 289.6, 177.9, 138.9, and 109.9, respectively (Figure S3). The RT for GC separation and MS pattern of the five characteristic peaks were in agreement with those of the authentic standards of MP, γ-HCH, 2,5-DCHQ, PNP, and HQ, which indicated that the characteristic peaks truly represented MP, γ-HCH, 2,5-DCHQ, PNP, and HQ.

When cells of *P. putida* KTU-9 were inoculated at OD_{600} = 0.05 in M9 minimal medium supplemented with MP or γ-HCH, 100 mg/L MP and 25 mg/L γ-HCH were completely mineralized within 24 and 60 h, respectively (Figure S4). Meanwhile, cells could utilize MP and γ-HCH as the sole source of carbon for growth and the cultures reached a maximum OD_{600} of 0.87 and 0.56 at 24 and 60 h, respectively (Figure 5).

In GC–MS analysis of the cultures grown on 100 mg/L MP, two peaks with a RT of 7.3 and 5.7 min appeared in GC separation, which were further identified as PNP and HQ according to their mass spectral properties. In GC–MS analysis of the cultures grown on 25 mg/L γ-HCH, two peaks appeared at a RT of 9.8 and 5.7 min in GC separation, which had the same MS patterns as 2,5-DCHQ and HQ (data not shown). These results indicated that MP and γ-HCH could be converted to HQ by the heterologous pathways assembled in *P. putida* KT2440 (Figure 1). HQ could be further transformed to β-ketoadipate, an intermediate in the tricarboxylic acid (TCA) cycle, by the intrinsic catabolic pathway in *P. putida* KT2440. Collectively, we concluded that the pathways for complete mineralization of MP and γ-HCH were functionally assembled in *P. putida* KT2440.

**Functional Expression of VHb and GFP.** As shown in Figure 6A, cells of *P. putida* KTU-9 containing *vgb* were more dark red-brown compared to cells of KTU without *vgb*. The biochemical activity of the VHb was tested using the CO-difference spectrum assay, based on the observation of a characteristic 420 nm absorption peak due to the formation of the CO–VHb complex. This peak was detected with cell-free extract of *P. putida* KTU-9 in the presence of CO (Figure 6B), which indicated that *vgb* was functionally expressed in *P. putida* KTU-9.

Expression of VHb in various hosts under oxygen-limited conditions has been shown to improve cell growth, protein secretion, and metabolite productivity. In this study, VHb-
expressing *P. putida* KTU-9 grew faster than *P. putida* KT2440 under oxygen-limited conditions (Figure S5). VHb may improve the ability of *P. putida* KTU-9 to sequester O₂ in soil during bioremediation, thus making this bacterium more competitive in soil microbial community.

Micrographs of GFP fluorescence taken on a confocal microscope indicated that GFP was present in an active form in *P. putida* KTU-9 (Figure S6). GFP may be a powerful real-time monitoring tool for the visualization of *P. putida* KTU-9 during bioremediation.

### Genetic Stability of Engineered Strain

To test whether inserted exogenous genes stably exist on the chromosome of *P. putida* KTU-9, strain KTU-9 was successively subcultured for 50 generations at 30 °C in LB medium. As a result, these subcultures still retained the mineralization capability for MP and γ-HCH, and the inserted exogenous genes were also detected by PCR from these subcultures, which demonstrated that the engineered strain was genetically stable, and that there was no need for selection pressure to maintain exogenous genes.

In this study, the cell growth of *P. putida* KTU-9 was compared to that of *P. putida* KTU. The two strains showed similar growth profiles during 48 h of incubation and reached the almost same cell density (Figure S7), indicating that the integration of nine exogenous genes into the chromosome had no negative effects on cell growth.

### Simultaneous Mineralization of MP and γ-HCH in Soil by *P. putida* KTU-9

To examine whether *P. putida* KTU-9 can thrive and effectively mineralize MP and γ-HCH in soil in the presence of the indigenous microbe populations, soils cocontaminated with MP and γ-HCH were inoculated with *P. putida* KTU-9 at the rate of 10⁶ cells/g. During the 11-day period, a slight reduction of MP and γ-HCH was observed in soils without inoculation, most likely due to the occurrence of abiotic degradation processes. In contrast, MP and γ-HCH were completely degraded in soils with inoculation within 6 and 11 days, respectively (Figure 7). However, the degradation rates for MP and γ-HCH were slower than those observed in liquid cultures, which may reflect the low bioavailability of MP and γ-HCH in soil. During the 11-day period, three key intermediates produced from degradation of MP and γ-HCH were also monitored. PNP was detected in soils in the first 8 days and thereafter PNP could not be detected. HQ and 2,5-DCHQ were detected in soils in the first 10 days; however, they could not be detected in soils on the 11th day (Figure S8). These results indicated that MP and γ-HCH were completely mineralized by the inoculated engineered strain in soils.

Soil samples were taken on the 11th day for the isolation of the inoculated *P. putida*. Three colonies that produced yellow halos on LB agar plates containing 50 mg/L MP were isolated. These three isolates, named SKT-1, SKT-2, and SKT-3, were identified as *P. putida* KT2440 by sequencing their 16S rRNA gene (sequencing results from strain SKT-1, Figure S9). Moreover, *mpd*, *pnpA*, *pnpB*, *gfp*, *linA*, *linB*, *linC*, *linD*, and *vgb* genes were detected by PCR from these three isolates (PCR results from strain SKT-1, Figure S10).

*P. putida* KTU-9
that the inoculated *P. putida* was truly responsible for the observed degradation.

**Potential of Synthetic Biology to Create Novel Strains for Bioremediation.** In 2010, a γ-HCH-mineralizing strain was constructed by introducing a dehydrochlorinase gene (*linA*) into the 1,2,4-trichlorobenzene-degrading *P. nitro-reducens* J5-1. To obtain a TCP-mineralizing organism, recently, a haloalkane dehalogenase gene was introduced into the genome of the 2,3-dichloro-1-propanol (DCP)-degrading *P. putida* MC4 using a transposon delivery system. However, in most cases, it is difficult to obtain such host strains with the desired degradation properties, and the biosafety of the strains needs to be further confirmed prior to practical application. An alternative strategy is to select a model strain with clear genetic background, confirmed biosafety, and well-established genetic manipulation techniques. In this study, *P. putida* KT2440 was used as a host strain for functional assembly of multiple catabolic pathways. The complete genome *P. putida* KT2440 has been sequenced and the chromosomal scarless modification methods have been well established for *P. putida* KT2440, which should pave the way for targeted chromosomal gene modification and metabolic pathway engineering in *P. putida* KT2440. Moreover, *P. putida* KT2440 is regarded as a safety strain, and possesses diverse catabolic pathways for aromatic compounds. These desirable traits make this bacterium a promising candidate for *in situ* bioremediation of soil.

In this study, no redundant sequences (e.g., antibiotic resistance gene) other than the inserted exogenous genes were left on the chromosome of *P. putida* KT2440. Therefore, the stable and unmarked engineered strain has enormous potential for use in bioremediation of soil cocontaminated with MP and γ-HCH. More importantly, the current strategy allows the successive unmarked insertions of various organic-degrading genes into the chromosome of *P. putida* KT2440 to create a

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**Figure 6.** Expression of *Vitreoscilla* hemoglobin (VHb) in *P. putida* KTU-9. (A) Impact of VHb expression on the color of bacterial cells. (B) CO-difference spectral analysis for *P. putida* KTU-9. *P. putida* KTU-9 showed a characteristic peak at 420 nm due to the binding of CO to VHb. *P. putida* KTU was used as the negative control.

**Figure 7.** Simultaneous degradation of methyl parathion (MP) and γ-hexachlorocyclohexane (γ-HCH, lindane) in soils inoculated with *P. putida* KTU-9 at the rate of 10⁶ cells/g soil. Symbols: (●) soil, inoculated; (■) soil, uninoculated. Data are mean values ± standard deviations from three replicates.
Table 1. Strains, Plasmids, and Primers Used in This Study

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<th>Strains, plasmid, or primer</th>
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<tr>
<td>KTU-9</td>
<td>KT2440 mutant (Δupp, ΔphaC1, ΔphaI&amp;G, Δfs, Δuvh, mpd, linA, linB, linC, linD, mpd+, pnpB, vgB+, gfP+)</td>
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P. putida strains were grown in LB medium or M9 minimal medium at 30 °C. E. coli strains were grown in LB medium at 37 °C. Media were supplemented with kanamycin (50 μg/mL) or S-FU (20 μg/mL) when required.

**Construction of P. putida KT2440 Mutants by Scarless Gene Replacement.** The strains, plasmids, and primers used in this study are listed in Table 1. A gene cassette containing the upstream and downstream homologous arms, a P. putida constitutive promoter J23119, a ribosomal binding site (RBS), and an OP-degrading gene (mpd), was chemically synthesized by BGI Inc., Beijing, China. The upp gene with its original promoter was obtained by PCR from the chromosome of P. putida KT2440 and then was cloned into the BamHI site of suicide plasmid pK18mob sacB to create pKU. The synthetic gene cassette was released with BamHI and HindIII from pUC57-Simple vector and then subcloned into the same restriction sites of pKU to generate the gene targeting vector pKU-1.

Plasmid pKU-1 was transformed into P. putida KTU using the electroporation method of Cho et al. and then integrated into the chromosome of P. putida. First, the recombinants occurring with the first crossover were selected on the LB plate...
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Table 2. Information on Nine Exogenous Genes and Their Chromosomal Insertion Sites

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Cells were inoculated at OD600 = 0.05 in 50 mL of M9 minimal medium. Cells were harvested by centrifugation, washed twice with 0.1 M potassium phosphate buffer (pH 7.2) twice, and then treated with 10 μM 5-FU for 12 h of incubation. Total RNA was prepared using a RNApure Bacteria kit (CWbio, Beijing, China). DNA contamination was checked by agarose gel electrophoresis. cDNA was obtained by reverse transcription with a HiFi-Reverse transcriptase and DNA integrity was checked by agarose gel electrophoresis. cDNA was obtained by reverse transcription with a HiFi-MMLV cDNA kit (CWbio). Subsequently, cDNA was used as a template for PCR amplification with a LA Taq DNA polymerase (Takara) on a Mastercycler gradient PCR thermal cycler (Eppendorf, Germany). PCR products were separated by electrophoresis at 80 V on 0.8% agarose gel.

**Detection of Transcription of the Inserted Genes by PCR.** Cells were cultured in LB medium and were harvested after 12 h of incubation. Total RNA was prepared using a RNApure Bacteria kit (CWbio, Beijing, China). DNA contamination was eliminated by a DNase I treatment at 37 °C for 1 h. RNA integrity was checked by agarose gel electrophoresis. cDNA was obtained by reverse transcription with a HiFi-MMLV cDNA kit (CWbio). Subsequently, cDNA was used as the template for PCR amplification with a LA Taq DNA polymerase (Takara) on a Mastercycler gradient PCR thermal cycler (Eppendorf, Germany). PCR products were separated by electrophoresis at 80 V on 0.8% agarose gel.

**Mineralization of MP and γ-HCH by P. putida KTU-9.** Cells of P. putida KTU-9 were cultured in LB medium at 30 °C for 12 h, harvested by centrifugation, and washed twice with M9 minimal medium. Cells were inoculated at OD600 = 0.05 in 50 mL of M9 minimal medium supplemented with 100 mg/L MP, 100 mg/L γ-HCH, 500 mg/L 2,5-DCHQ, 500 mg/L PNP, or 500 mg/L HQ in 250-mL flasks and grown at 200 rpm and 30 °C. Degradation experiments with wild-type P. putida KT2440 were performed under the same conditions. Aliquots (1 mL) of samples were withdrawn at regular time intervals.

Extraction of the tested chemicals from liquid samples and analysis for their concentration by gas chromatography–mass spectrometer (GC–MS) were performed as described below.

**CO–Difference Spectral Analysis.** VHb activity was detected by CO-difference spectra. Hemoglobin is known to react with CO. The complex consisting of carbon monoxide and reduced hemoglobin has a characteristic peak at 420 nm. Cells were harvested by centrifugation at 4000 rpm and 4 °C for 10 min after 24-h cultivation in LB medium. Cell pellets were washed with 0.1 M potassium phosphate buffer (pH 7.2) twice. Cells were resuspended in 20 mL of the same buffer and were disrupted with a sonicator on ice. The crude extract was centrifuged at 10 000 rpm and 4 °C for 20 min to remove cell debris. Hemoglobin levels in cell-free extracts were detected by CO-difference spectra using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

**Imaging Bacteria.** Cells were grown to mid-log phase (OD600 = 0.6) at 30 °C, harvested by centrifugation, washed with PBS buffer (pH 7.2) twice, and then treated with 10 μM FM4-64/L dye for 15 min. Cells were fixed with 2% glutaraldehyde on a slice. Cells were observed with a Leica TCS SP5 confocal microscope, fitted with a Leica 100 × 10 numerical aperture objective lens, using parameters appropriate for the fluorescence excitation. Expression levels of the GFP were reliably predicted from the fluorescence intensity.

**Soil Remediation Experiments.** Soil samples used in this study were collected from the campus of Nankai University, Tianjin, China. The soil samples were never exposed to any pesticides before. The soil had a pH of 6.8. Subsamples (100 g) of the soil were treated under aerobic condition with MP (50 mg/kg soil) and γ-HCH (25 mg/kg soil). Soil samples in triplicate were inoculated with P. putida KTU-9 (108 cells/g, an inoculum density recommended by Singh et al.24) and triplicate soil samples without inoculation were kept as the controls. The inoculum was thoroughly mixed into the soils under sterile condition. The soil moisture was adjusted by the addition of distilled water to 40% of its water-holding capacity. The soils were incubated at 30 °C for 11 d in the dark. Soil samples (5 g) were withdrawn at different time intervals for the quantification of MP, γ-HCH, PNP, 2,5-DCHQ, and HQ. Extraction of these chemicals from the soil was carried out using the previous methods described in Singh et al.24 and Bidlan et al.25 Analysis for the concentration of these chemicals was performed by GC–MS as described below.
GC–MS Analysis. The residual pesticides and aromatics in liquid samples were extracted twice with the mixture of hexane and acetone (1:1, v/v) and then extracted once with ethyl acetate. The organic layers were pooled and then dried over anhydrous Na₂SO₄. Samples of 1 µL (diluted if necessary) were injected directly for GC–MS analysis, which was obtained on an Agilent Technologies 7890A-5975C (Agilent Technologies, Palo Alto, CA, USA). The GC was equipped with a HP-5 capillary column (29.9 m x 0.25 mm x 0.25 µm) and operated in splitless mode. Helium (>99.999%) was used as carrier gas with a constant flow rate of 1 mL/min. The GC conditions were as follows: injector temperature of 220 °C, oven temperature increasing from 70 to 320 °C at 10 °C/min, and interface temperature of 250 °C. The mass spectrum conditions were as follows: ionization energy of 70 eV, scan range of 30–300 amu, and ion chamber temperature of 250 °C. The mass spectra of the intermediates were determined by using a standard curve of peak area versus concentration. For product identification, the mass spectra of the intermediates were compared with those of their authentic standards or the same compound available in the National Institute of Standard Technology (NIST) library, USA.

■ ASSOCIATED CONTENT

1 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.6b00025.

Figure S1: (A) scheme of upp-based counterselection system for P. putida KT2440; (B) assembly of exogenous genes and their expression regulation elements. Figure S2: nucleotide sequences of DNA extracted from the amplified bands of lanes 1–9 in Figure 2. Figure S3: GC–MS analysis for degradation of MP, γ-HCH, 2,5-DCHQ and HQ by P. putida KTU-9. Figure S4: mineralization of MP and γ-HCH by P. putida KTU-9. Figure S5: time courses for the growth of P. putida KTU-9 and KT2440 under oxygen-limited conditions. Figure S6: detection of GFP fluorescence derived from P. putida KTU-9 using a confocal microscope. Figure S7: time courses for the growth of P. putida KTU-9. Figure S8: quantification of PNP, 2,5-DCHQ and HQ produced from degradation of MP and γ-HCH in soils inoculated with P. putida KTU-9 at the rate of 10⁶ cells/g soil. Figure S9: sequencing results of the 16S rRNA gene of strain SKT-1. Figure S10: PCR detection of mpd, pnpA, pnpB, gfp, linA, linB, linC, linD and vgb genes in strain SKT-1. Figure S11: nucleotide sequences of the synthetic gene cassettes. Supplementary methods: construction of P. putida KTU-4, KTU-6 and KTU-9 by scarfless gene replacement (PDF)

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Notes

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■ ABBREVIATIONS USED

OP, organophosphates; OC, organochlorines; MP, methyl parathion; γ-HCH, γ-hexachlorocyclohexane; 2,5-DCHQ, 2,5-dichlorohydroquinone; PNP, p-nitrophenol; HQ, hydroquinone; 5-FU, 5-fluouracil; Kan, kanamycin; MPH, methyl parathion hydrolyase; LinA, gamma-BHC dechlorinase; LinB, haloalkane dehalogenase; LinC, 2,5-dichloro-2,5-cyclohexadiene-1,4-diodehydrogenase; LinD, reductive dechlorinase of 2,5-dichlorohydroquinone; pnpA, p-nitrophenol 4-monoxygenase; pnpB, 1,4-benzoquinone reductase; VhB, Vitreoscilla hemoglobin; GFP, green fluorescent protein; GC-MS, gas chromatography–mass spectrometer

■ REFERENCES


