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ORIGINAL PAPER

Simultaneous degradation of organophosphate and organochlorine pesticides by *Sphingobium japonicum* UT26 with surface-displayed organophosphorus hydrolase

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Abstract A genetically engineered microorganism (GEM) capable of simultaneously degrading organophosphate and organochlorine pesticides was constructed for the first time by display of organophosphorus hydrolase (OPH) on the cell surface of a hexachlorocyclohexane (HCH)-degrading *Sphingobium japonicum* UT26. The GEM could potentially be used for removing the two classes of pesticides that may be present in mixtures at contaminated sites. A surface anchor system derived from the truncated ice nucleation protein (INPNC) from *Pseudomonas syringae* was used to target OPH onto the cell surface of UT26, reducing the potential substrate uptake limitation. The surface localization of INPNC–OPH fusion was verified by cell fractionation, western blot, proteinase accessibility, and immunofluorescence microscopy. Furthermore, the functionality of the surface-exposed OPH was demonstrated by OPH activity assays. Surface display of INPNC–OPH fusion (82 kDa) neither inhibited cell growth nor affected cell viability. The engineered UT26 could degrade parathion as well as γ-HCH rapidly in minimal salt medium. The removal of parathion and γ-HCH by engineered UT26 in sterile and non-sterile soil was also studied. In both soil samples, a mixture of parathion (100 mg kg^{-1}) and γ-HCH (10 mg kg^{-1}) could be degraded completely within 15 days. Soil treatment results indicated that the engineered UT26 is a promising multifunctional bacterium that could be used for the bioremediation of multiple pesticide-contaminated environments.

Keywords Organophosphate · Organochlorine · Organophosphorus hydrolase · *Sphingobium japonicum* UT26

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Introduction

Synthetic organophosphates (OPs) have played an important role in raising agricultural productivity and controlling pests (Singh and Walker 2006; Singh 2009). Organophosphates are acute neurotoxins

because these compounds irreversibly inhibit acetylcholinesterase in the central nervous system synapses of vertebrates, including humans, leading to a subsequent loss of nerve function and eventual death (Sogorb et al. 2004). Organophosphates are mostly liposoluble and pose a hazard to humans through accumulation in the food chain, which leads to delayed cholinergic toxicity. The technical formulation of hexachlorocyclohexane (t-HCH), a recalcitrant organochlorine pesticide, mainly consists of α-, β-, γ- and δ-isomers (Willett et al. 1998). Among these four isomers, only γ-HCH (also called γ-BHC or lindane) has insecticidal activity and has been used worldwide to control a wide range of agricultural, horticultural, and public health pests. However, serious environmental problems have resulted from HCH usage, since all its isomers are toxic, highly persistent in the environment and tend to accumulate in biological tissues (Li et al. 2003).

Some microorganisms capable of degrading organophosphorus pesticides have been isolated, and an organophosphorus hydrolyse gene (*opd*) has been characterized (Singh and Walker 2006). Organophosphorus hydrolases (OPHs), isolated from natural soil microorganisms *Pseudomonas diminuta* MG and *Flavobacterium* sp. strain ATCC 27551, have been shown to hydrolyze different organophosphorus pesticides as well as the more toxic chemical-warfare agents such as Sarin, Soman, and VX (Serdar and Gibson 1985; Mulbry et al. 1986). Organophosphorus hydrolase is a zinc-containing homodimeric protein (36 kDa for each subunit). Organophosphates with a PNP substitution (e.g. paraoxon and parathion) are hydrolyzed by OPH to *p*-nitrophenol (PNP). Hydrolysis of OPs by OPH reduces their toxicity by several orders of magnitude (Sogorb et al. 2004).

Gram-negative bacteria possess a complex cell envelope structure that consists of a cytoplasmic membrane, cell wall, and outer membrane. The outer membrane prevents OPs from interacting with OPH residing within the cell, reducing the overall catalytic efficiency (Richins et al. 1997). This bottleneck, however, could be eliminated if OPH is displayed on the cell surface. So far, OPH has been displayed on the cell surface of *E. coli* (Richins et al. 1997), *Moraxella* sp. (Shimazu et al. 2001), *P. putida* (Lei et al. 2005) and *Saccharomyces cerevisiae* (Takayama et al. 2006).

Various surface-anchoring motifs have been developed to target heterologous proteins onto the cell surface, such as Lpp-OmpA, ice nucleation protein (INP), and autotransporter (Samuelson et al. 2002). The INP, which confers on host cells the ability to nucleate crystallization in supercooled water, is an outer membrane protein of *P. syringae* (Kozloff et al. 1991; Wolber 1993). The INP has a multidomain organization with an N-terminal domain containing three or four transmembrane spans, a C-terminal domain, and a highly repetitive central domain for ice nucleation. INP-mediated cell surface display can be achieved using either full-length INP sequences or truncated INP sequences (Jung et al. 1998; Shimazu et al. 2001; Li et al. 2004).

Many γ-HCH-degrading bacteria such as *Sphingobium japonicum* UT26 from Japan (Senoo and Wada 1989), *Sphingobium indicum* B90 from India (Kumari et al. 2002) and *Sphingobium francense* Sp + from France (Ceremonie et al. 2006), have been isolated and characterized. Despite a number of bacteria known to decompose γ-HCH, the degradation pathway has been elucidated comprehensively only in UT26, a strain isolated from an upland experimental field in Japan where γ-HCH had been applied once a year for 12 years (Phillips et al. 2005; Lal et al. 2006). UT26 utilizes γ-HCH as a sole source of carbon and energy under aerobic conditions (Imai et al. 1989). The enzymes and genes involved in the degradation pathway of γ-HCH in UT26 have been elucidated. The primary enzyme for γ-HCH degradation in UT26 is HCH dehydrochlorinase, encoded by only one copy of the gene *linA* (Nagata et al. 1993). The remaining genes of the γ-HCH degradation pathway, i.e., *linB*, *linC*, *linD*, *linE*, *linF*, *linR*, and *linX*, encoding a halidohydrolase, a dehydrogenase, a reductive dechlorinase, a dioxygenase, a reductase, a LysR-type transcriptional regulator, and another dehydrogenase, respectively, have been cloned and characterized from UT26 (Nagata et al. 2007; Lal et al. 2010).

Because of the various kinds of pesticide-residues present in the environment, multifunctional genetically engineered microorganisms (GEMs) are needed to clean up these pollutants (Chen et al. 1999). So far, specific microbes with the capability to degrade both OPs and HCH have not been isolated from the natural environment. In this study, OPH was functionally displayed on the cell surface of the HCH-degrading UT26 using the INP anchoring system, resulting in a GEM capable of simultaneously degrading HCH and OPs.

Materials and methods

Bacterial strains, plasmids, and culture conditions

Sphingobium japonicum UT26 was grown on 1/3 Luria–Bertani (LB) medium (3.3 g of Bacto Tryptone, 1.7 g of yeast extract and 5 g of sodium chloride, per liter) or minimal salt medium (MSM) (2.0 g Na_2HPO_4, 0.75 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 1.0 g NH_4Cl per liter, pH 7.0) supplemented with 100 mg l^{-1} parathion or 10 mg l^{-1} γ-HCH added if needed at 30 °C. All chemicals were purchased from Sigma Co. A surface expression vector, pPNCO33 (Shimazu et al. 2001), coding for INPNC–OPH was used to target OPH onto the cell surface. The *inpnc–opd* fragment was PCR amplified from pINCOP and subcloned into *Eco*RI/*Hin*dIII-digested pVLT33 (de Lorenzo et al. 1993), an *E. coli–Pseudomonas* shuttle vector, to generate pPNCO33. Plasmid pCPO (Liu et al. 2009) was used for the production of cytosolic OPH.

Transformation of pPNCO33 into UT26 was performed using the electroporation method of Grag et al. (1999). After the cultures were grown to an optical density (OD_{600}) of 0.4–0.6, cells were harvested and placed on ice for 30 min. Cells were then washed four times with ice-cold sterile deionized water and recovered by centrifugation at 9,000 *g* for 10 min at 4 °C. After an additional wash with 10 % sterilized ice-cold glycerol, cells were resuspended in 10 % glycerol at 10^{10}–10^{11} cells/ml and 90 µl of cell suspension was mixed with 2 µg of plasmid DNA. After vortexing for 10 s, the sample was kept on ice for 30 min before electroporation (2.5 kV, 25 µF, 200 Ω, BioRad). Transformed cells were incubated in LB medium for 24 h and cells were selected on LB agar plates containing 50 µg ml^{-1} of kanamycin.

Expression of INPNC–OPH was induced with 0.2 mM (isopropyl-β-D-thiogalactopyranoside) IPTG for 24 h at 30 °C when cells were grown to an optical density at 600 nm (OD_{600}) of 0.4.

Cell fractionation

Cells were harvested and resuspended in 25 mM Tris–HCl buffer (pH 8.0). After disruption of the cells by sonication (BioRad) and a brief clarifying spin, the clarified lysate was ultracentrifuged (Beckman) at 50,000 rpm for 1 h at 4 °C and the supernatant was retained as the soluble fraction. The pellet (total membrane fraction) was resuspended with PBS containing 0.01 mM $MgCl_2$ and 2 % Triton X-100 for solubilizing the inner membrane and was incubated for 30 min at room temperature, and then the outer membrane fraction was repelleted by ultracentrifugation (Lei et al. 2005).

SDS-PAGE and western blot analysis

Subcellular fractionated samples were mixed with sample buffer (200 mM Tris–HCl, pH 6.8, 8 % SDS, 0.04 % bromophenol blue, 8 % β-mercaptoethanol, 40 % glycerol), boiled for 5 min, and analyzed by 12 % sodium dodecyl sulfate-polyacyrlamide gel electrophoresis (SDS-PAGE) (Sambrook and Russel 2001). After electrophoresis, the separated proteins were electroblotted overnight at 40 V to the nitrocellulose membrane (Millipore) with a tank transfer system (BioRad) containing a transfer buffer (25 mM Tris, 192 mM glycine, 10 % methanol). After blocking nonspecific binding sites with 3 % bovine serum albumin (BSA) in TBST buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.05 % Tween-20), the membrane was incubated with rabbit anti-OPH serum (a gift from Prof. Ashok Mulchandani) at a 1:1,000 dilution in TBST buffer for 3 h. Subsequently, the membrane was incubated with alkaline phosphatase-conjugated goat anti-rabbit IgG secondary antibody (Promega) at a 1:2,000 dilution in TBST buffer for 2 h. The membrane was then stained with Nitro Blue Tetrazolium–BCIP (5-bromo-4-chloro-3-indolylphosphate) in alkaline phosphatase buffer (100 mM Tris–HCl, pH 9.0, 100 mM NaCl) for visualization of antigen-antibody conjugates.

Immunofluorescence microscopy

Cells were harvested and resuspended ($OD_{600} = 0.5$) in PBS with 3 % BSA. Cells were then incubated with rabbit anti-OPH serum diluted (1:500) in PBS for 3 h at 30 °C. After being washed with PBS, the cells were resuspended in PBS with goat anti-rabbit IgG antibody conjugated with rhodamine (1:100 dilution, Invitrogen) and incubated for 2 h at 30 °C. Prior to microscopic observation, cells were washed five times with PBS and mounted on poly(L-lysine)-coated microscopic slides. Photographs were taken using a fluorescence microscope (Nikon) equipped with FITC and rhodamine filters.

OPH activity assay

OPH activity was assayed with paraoxon by monitoring the increases in the absorbance at 405 nm (ε_{405} = 17,700 M^{-1} cm^{-1} for *p*-nitrophenol) for 2 min at 37 °C using a Beckman DU800 spectrophotometer (Richins et al. 1997). For each assay, 200 µl of cells (OD_{600} = 1.0) were added to 700 µl of 50 mM citrate–phosphate buffer with 50 µM $CoCl_2$ (pH 8.0) and 100 µl of 20 mM paraoxon (Sigma) in 10 % methanol. Activities are expressed as units (1 µmol of *p*-nitrophenol produced per minute) per OD_{600} of whole cells.

Proteinase accessibility assay

Cells were centrifuged and resuspended (OD_{600} = 1.0) in 1 ml of 15 % sucrose, 15 mM Tris–HC1, and 0.1 mM EDTA, pH 7.8. Samples were incubated for 3 h with 5 µl of 20 mg ml^{-1} proteinase K at room temperature. Proteinase K treated cells were assayed for OPH activity, western blot and immunofluorescence microscopy as described above.

Stability study of resting cultures

Cells were grown in 50 ml of LB medium supplemented with 0.2 mM IPTG and 50 µg ml^{-1} kanamycin for 2 days, washed twice with 50 ml of 150 mM NaCl solution, resuspended in 5 ml of 50 mM citrate–phosphate buffer with 50 µM $CoCl_2$ (pH 8.0), and incubated in a shaker at 30 °C. Over a 2-week period, 0.1 ml of samples was removed each day. Samples were centrifuged and resuspended in 0.1 ml of 50 mM citrate–phosphate buffer (pH 8.0) containing 50 µM $CoCl_2$. OPH activity assays were conducted as described above.

Biodegradation experiments

Unless stated otherwise, the pesticides biodegradation experiments in the study were carried out as below: the engineered UT26 (or native UT26) was precultured in 1/3 LB medium at 30 °C and harvested during log phase, respectively. The cultures were centrifuged and the cell pellets were washed twice with fresh MSM (pH 7.0) and used as inoculums. Then, 10^6 cells ml^{-1} were inoculated into MSM supplemented with 50 mg l^{-1} kanamycin, 0.1 % glucose, 100 mg l^{-1} parathion and 10 mg l^{-1} γ-HCH. Cultures were maintained in 250 ml bottles at 30 °C and 200 rpm on a shaker. Appropriate controls containing medium plus γ-HCH and parathion were kept simultaneously.

Aliquots (1 ml) were removed periodically and extracted twice with 4 ml of ethyl acetate for parathion and hexane for γ - HCH. These extracts were dehydrated with Na_2SO_4 and filtered (0.45 µm). Samples of 1 µl (diluted if necessary) were analyzed using a Hewlett-Packard 5890 II gas chromatography equipped with ECD detector and an OV-1701 GC column (polydimethylsiloxane 25 m × 0.32 mm × 0.25 µm). The column, injector, and detector temperatures were maintained at 210, 250, and 300 °C, respectively, with a flow rate of 5.4 ml min^{-1}. The concentration was determined by comparing peak area of the samples to a standard curve.

The soil samples were from the campus of Nankai University, Tianjin, China, and were never exposed to any HCH and OPs pesticides before. The soil had a pH of 6.82 and its organic carbon was 5.28 g kg^{-1}. Soil samples (5 kg) were sterilized by fumigation with chloroform for 10 days at 30 °C (Singh et al. 2004). Subsamples (100 g) of the fumigated and nonfumigated soil were treated under aseptic condition with parathion (100 mg kg^{-1}) and γ-HCH (10 mg kg^{-1}), respectively. One set of fumigated and nonfumigated soils in triplicate was inoculated with the engineered UT26 (10^6 cells g^{-1}), and another set without inoculation was kept as 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 15 days in the dark. Extraction of parathion and γ-HCH from the soil was carried out by the method described in Singh et al. (2004) and Bidlan et al. (2004). Analysis for parathion and γ-HCH residues was performed by gas chromatography as described above.

Results and discussion

Surface localization of INPNC–OPH in UT26

Both *InaK* from *P. syringae* KCTC1832 and *InaV* from *P. syringae* INA5 have been used for the display of heterologous proteins on the cell surface (Schmid et al. 1997; Jung et al. 1998). Even though functionally similar, there is only 77 % sequence homology between the two proteins. Most of the differences

occur at the critical N-terminal domain, which interacts with the phospholipid moiety of the outer membrane. Compared to the *InaK* anchor, the use of the *InaV* anchor resulted in 100-fold higher OPH activity in *Moraxella* sp. (Shimazu et al. 2001). In the present study, to investigate the feasibility of targeting OPH onto the cell surface of UT26, the truncated *InaV* protein (INPNC) (Schmid et al. 1997) was used as a surface-anchoring motif. For expression of INPNC–OPH, the *inpnc–opd* fragment was subcloned into pVLT33, a medium-copy-number vector, to create pPNCO33. The broad-host-range vector, pVLT33, is an RSF1010 derivative, and therefore, is able to replicate in a wide variety of Gram-negative bacteria (de Lorenzo et al. 1993). Expression of INPNC–OPH was tightly regulated by a *tac* promoter due to the presence of the $lacI^q$ gene on the plasmid.

Expression of INPNC–OPH in UT26 was verified by western blot with anti-OPH serum. A band corresponding to INPNC–OPH at 82 kDa was detected in whole-cell lysates (Fig. 1, lane 5). However, no such protein was detected with the control cells carrying pVLT33. To assess the distribution of INPNC–OPH fusion between the outer membrane and soluble fractions, outer membrane and soluble fractions were probed with anti-OPH serum. More than 80 % of INPNC–OPH fusion was associated with the outer membrane fraction as judged by the intensity of the protein band (Fig. 1, lane 1). Subcellular fractionated samples were measured for the OPH activity. Over 80 % of OPH activity was detected in the outer membrane fraction of cells expressing INPNC–OPH. In parallel, more than 80 % of OPH activity was present on the cell surface as judged from the ratio of whole-cell activity to cell lysate activity (Table 1).

Proteinase accessibility experiments were performed to ascertain the surface localization of INPNC–OPH fusion. Since proteinase cannot readily penetrate the cell membrane, degradation should occur only with proteins exposed on the cell surface (Richins et al. 1997). With the proteinase treatment, the OPH activity of UT26/pPNCO33 cells decreased by 83 %, while the OPH activity of UT26/pCPO cells expressing cytosolic OPH dropped only 6 %. After the treatment of cells with proteinase K, the fractionated outer membrane samples were probed with anti-OPH serum. As expected, no target proteins were detected in the outer membrane fraction (Fig. 1, lane 2) because of the degradation of surface-exposed OPH by proteinase K.

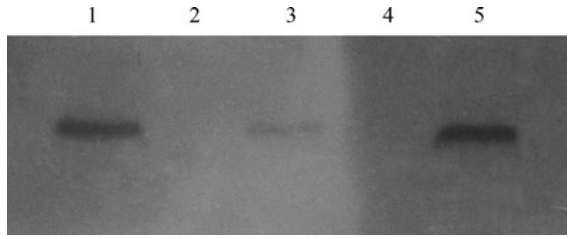

Fig. 1 Western blot analysis for subcellular localization of INPNC–OPH in *Sphingobium japonicum* UT26 harboring pPNCO33. *Lane 1*, outer membrane fraction; *lane 2*, proteinase K digested outer membrane proteins; *lane 3*, soluble fraction; *lane 4*, negative control (UT26 harboring pVLT33); *lane 5*, whole-cell lysates. OPH antiserum was used at a 1:1,000 dilution

Immunolabeling with specific antibodies or antisera is a useful tool to detect surface-exposed proteins (Li et al. 2004). To confirm the presence of INPNC–OPH on the cell surface, cells were probed with rabbit anti-OPH serum as a primary antibody and then fluorescently stained with rhodamine-labeled goat anti-rabbit IgG antibody. Since antibodies cannot diffuse through the outer membrane, specific interactions should only occur with proteins exposed on the cell surface. Under a fluorescence microscope, cells expressing INPNC–OPH were brightly fluorescent, while control cells expressing cytosolic OPH were not immunostained at all (data not shown).

Cells expressing INPNC–OPH were incubated for 3 h with proteinase K and then immunolabeled with anti-OPH serum and rhodamine-conjugated IgG secondary antibody. As a result, proteinase K treated cells were not immunostained completely, indicating that surface-exposed OPH had been removed after the treatment with proteinase K. From all of these results, we concluded that OPH was displayed functionally on the cell surface using the INPNC anchor.

OPH activity

The whole-cell OPH activity of UT26/pPNCO33 cell displaying OPH was sevenfold higher than that of UT26/pCPO cell expressing cytosolic OPH. The surface-exposed OPH proteins have free access to the extracellular OP substrates; thus, this enhances whole-cell catalytic efficiency. The OPH activity of cell lysate and whole cell reached maximums at 12 h and 24 h, respectively (Fig. 2). Time course of OPH activity suggests that stationary-phase translocation of the previously synthesized INPNC–OPH fusion may

Table 1 Percentage of surface-exposed OPH of UT26 cell harboring pPNCO33 or pCPO as estimated from proteinase K treatment, whole cell versus lysate assays, and membrane fractionation experiments

Plasmid	% Decrease in activity in proteinase K treated cells	Whole-cell activity (as % of cell lysate activity)	% Activity in outer membrane fraction
pPNCO33	83	84	81
pCPO	6	5	3

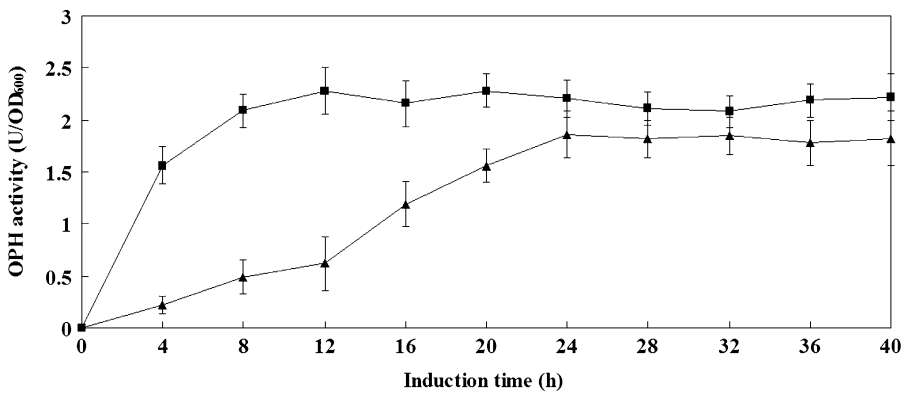

Fig. 2 Time course analysis of the OPH activity of *Sphingobium japonicum* UT26 expressing INPNC–OPH. Cells were incubated at 30 °C for 40 h after induction with 0.2 mM IPTG. The OPH activity of whole cell (*filled triangle*) and total cell lysate (*filled square*) was determined using paraoxon as the substrate. The data are means ± standard deviations of three replicates

contribute to the rapid increase of whole-cell OPH activity after 12 h.

Secretion is generally the limiting step for a secretory protein; thus, a high transcription rate may block the translocation pathway and cause growth inhibition of cells (Rodrigue et al. 1999). The inhibitory effects of overexpression on the translocation pathway have been reported in previous studies with surface expression of green fluorescent protein (Shi and Su 2001; Li et al. 2004). In order to achieve a high level of whole-cell activity, it is important to investigate the optimal levels of expression of OPH. Here, effects of different levels of induction on activity were studied. The expressed INPNC–OPH was located in the outer membrane fraction when IPTG induction was performed at a concentration of 0.2 mM. However, the fusion proteins produced with induction at higher IPTG concentrations (0.5 and 1 mM) were present only in the soluble fraction. In this study, whole-cell activity reached a maximum at an IPTG concentration of 0.2 mM (Fig. 3). Further induction resulted in a gradual decline in activity. Our results suggest that INPNC–OPH fusion cannot be efficiently targeted onto cell surfaces under high levels of induction.

Stability of UT26 cell displaying INPNC–OPH fusion

Anchorage of foreign proteins on the outer membrane may result in instability of the outer membrane and growth inhibition of the cells (Samuelson et al. 2002). To test whether surface display of OPH inhibits cell growth, the growth kinetics of cells carrying pPNCO33 or pVLT33 were compared. No growth inhibition was observed for cells expressing INPNC–OPH. The two cultures reached the same final cell density after 48 h of incubation. To monitor the stability of suspended cultures, whole-cell activity was determined periodically over a 2-week period. The OPH activity of whole cells remained at essentially the original level over the 2-week period. These results show that surface display of OPH did not disturb the membrane structure or cause host growth defects.

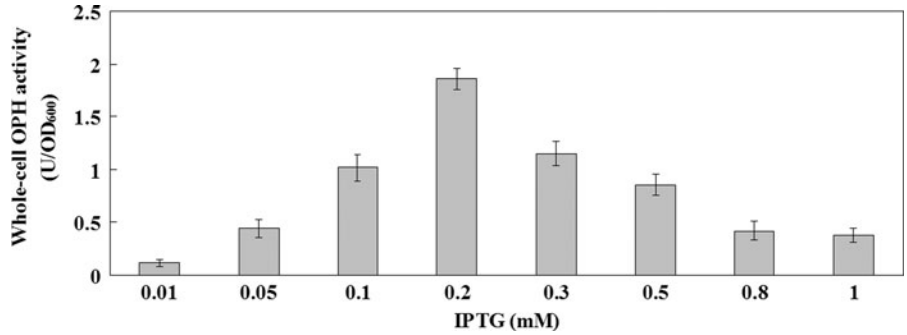

Fig. 3 Whole-cell OPH activity of *Sphingobium japonicum* UT26 harboring pPNCO33 under different levels of induction. The activity was assayed with paraoxon as the substrate. The data are means ± standard deviations of three replicates

Simultaneous degradation of γ-HCH and parathion by engineered UT26 in MSM

In order to determine the degradation rate of parathion and γ-HCH, the engineered UT26 (or native UT26) was inoculated into the MSM containing 100 mg l^{-1} parathion and 10 mg l^{-1} γ-HCH. Aliquots (1 ml) were removed periodically, extracted and analyzed by GC–ECD. Compared to native UT26, the engineered UT26 could degrade γ-HCH at the same rate, indicating that surface display of INPNC–OPH fusion did not affect the degradation capabilities of UT26 for γ-HCH. The γ-HCH (10 mg l^{-1}) was degraded to an undetectable level by engineered and native UT26 within 32 h. The rates became much slower from 20 to 32 h possibly because of the accumulation of intermediates of γ-HCH degradation. Parathion (100 mg l^{-1}) was completely degraded via hydrolysis of the phosphotriester bond in 24 h by engineered UT26, however, the concentration of parathion remained almost unchanged in the culture of native U26, which indicated that UT26 acquired the degradation capabilities for OPs by the surface expression of OPH. These results indicated that the engineered UT26 can be employed for the degradation of a mixture of pesticides containing OPs and HCH.

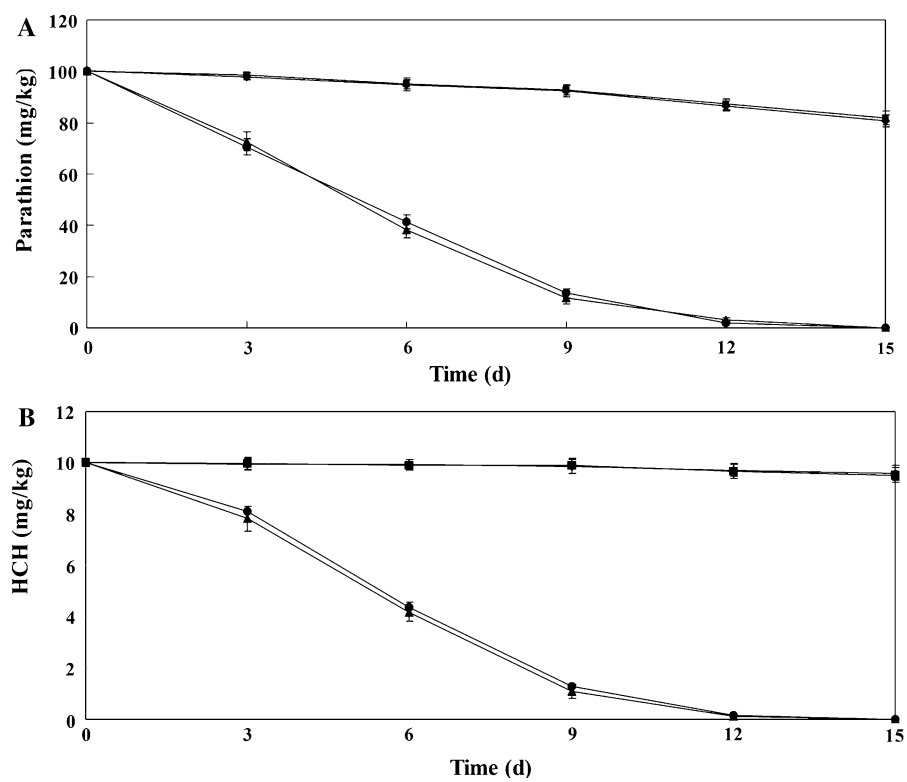

Fig. 4 Simultaneous degradation of parathion (**a**) and γ-HCH (**b**) by engineered UT26 at an initial rate of 10^6 cells g^{-1} in soil containing 100 mg kg^{-1} parathion and 10 mg kg^{-1} γ-HCH at 30 °C (*filled circle*, fumigated soil inoculated; *filled triangle*, nonfumigated soil inoculated; *filled diamond*, fumigated soil uninoculated; *filled square*, nonfumigated soil uninoculated). The data are means ± standard deviations of three replicates

Simultaneous degradation of γ-HCH and parathion by engineered UT26 in soil

To determine whether UT26 can degrade parathion and γ-HCH in soil when in competition with indigenous microbes, fumigated and nonfumigated soil were inoculated with the engineered UT26 at the rate of 10^6 cells g^{-1}. Both parathion (100 mg kg^{-1}) and γ-HCH (10 mg kg^{-1}) could be degraded completely within 15 days in fumigated and nonfumigated soil samples with inoculation (Fig. 4). Degradation rates of both pesticides in fumigated soils with inoculation were similar to those of nonfumigated soils. In contrast, less than 20 % and of parathion and less than 5 % of γ-HCH were degraded in control soils without inoculation in 15-day incubation studies (Fig. 4), most likely due to the chemical reaction of pesticides. Our studies on lab-scale soil bioremediation highlight the potential of the engineered UT26 for the removal of HCH and OP residues in the natural environment. In practical field-scale bioremediation, the effectiveness depends on many factors, i.e., the degrading capability of the strain, the adaptability of the strain to the fluctuating environmental conditions, competition with indigenous microbial populations and the bioavailability of contaminants (Bidlan et al. 2004; Singh et al. 2004).

In this study, we constructed a GEM with the capability to rapidly degrade both HCH and OPs, which offers a strong platform toward the successful utilization of this environmentally robust bacterium for the bioremediation of soils contaminated with multiple pesticides. Further studies should be conducted to examine the interactions of the soil environment with the strain and the effects of multiple soil parameters on degradation. This will be useful for the application of the strain in the field-scale bioremediation of HCH- and OPs-contaminated sites in the future.

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