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RESEARCH ARTICLE

Isolation of monocrotophos-degrading strain *Sphingobium* sp. YW16 and cloning of its Tn*opdA*

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Abstract The bacterial strain *Sphingobium* sp. YW16, which is capable of degrading monocrotophos, was isolated from paddy soil in China. Strain YW16 could hydrolyze monocrotophos to dimethylphosphate and *N*-methylacetoacetamide and utilize dimethylphosphate as the sole carbon source but could not utilize *N*-methylacetoacetamide. Strain YW16 also had the ability to hydrolyze other organophosphate pesticides. A fragment (7067 bp) that included the organophosphorus hydrolase gene, *opdA*, was acquired from strain YW16 using the shotgun technique combined with SEFA-PCR. Its sequence illustrated that *opdA* was included in Tn*opd*A, which consisted of a transpose gene, a putative integrase gene, a putative ATP-binding protein gene, and *opdA*. Additionally, a conjugal transfer protein gene, *traI*, was located downstream of Tn*opd*A. The juxtaposition of Tn*opd*A with TraI suggests that *opdA* may be transferred from strain YW16 to other bacteria through conjugation. OpdA was able to hydrolyze a wide range of organophosphate pesticides, with the hydrolysis efficiency decreasing as follows: methyl parathion > fenitrothion > phoxim > dichlorvos > ethyl parathion > trichlorfon > triazophos > chlorpyrifos > monocrotophos > diazinon. This work provides the first report of *opdA* in the genus *Sphingobium*.

Keywords Monocrotophos · Biodegradation · *Sphingobium* sp. YW16 · Tn*opd*A

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Introduction

Monocrotophos (MCP) is an organophosphorus insecticide (OP) that exhibits a relatively broad spectrum of activity. Humans have used this insecticide extensively since the 1960s to control foliar insects in cotton fields, vegetable crops, pastures, and paddy fields. Soil degradation of MCP includes chemical hydrolysis and microbial activity (Beynon et al. 1973; Gundi and Reddy 2006). Aerobic bacteria hydrolyze MCP to dimethylphosphate and *N*-methylacetoacetamide (MMAA) in most cases (Bhadbhade et al. 2002b). *O*-Demethylation, *N*-demethylation, cleavage of phosphate-crotonamide linkages, and hydroxylation of *N*-methyl groups occur during MCP metabolism by animals (Mucke 1994), plants (Skripsky and Loosli 1994), and microbes (Bhadbhade et al. 2002b; Guth 1994). O-Desmethyl monocrotophos, dimethylphosphate, monomethyl phosphate, *N*-methylacetoacetamide (MMAA), and *N*-methylbutyramide are formed during these processes. Bioremediation based on microorganisms is a more promising and effective method than traditional physicochemical methods for the removal of environmental pollutants (Dureja 1989; Kaur and Sud 2011; Ku et al. 1998; Madhavan et al. 2010), which are noted for their low efficiencies and high operating costs. Increasing evidence indicates that various microorganisms which belong to different taxa can effectively eliminate xenobiotic compounds (Chen et al. 2014; Gu et al. 2014; Zhang et al. 2011). Several effective MCP-degrading strains have been

previously described, including *Pseudomonas aeruginosa*, *Clavibacter michiganensis* (Singh and Singh 2003), *Bacillus megaterium*, *Arthrobacter atrocyaneus*, *Pseudomonas mendocina* (Bhadbhade et al. 2002a, b), and *Paracoccus* sp. M1 (Jia et al. 2006). However, these strains commonly degrade only a few types of OPs, which restricts their use in bioremediation, as a single region is commonly contaminated by a variety of OPs. In recent years, various gene/enzyme systems involved in the degradation of organophosphates have been reported. Organophosphorus pesticide degradation genes (*opd*/*opdA*) have been isolated from multiple organisms in different geographic locations (Horne et al. 2002a; Siddavattam et al. 2003). The *opd*/*opdA* gene encodes organophosphorus hydrolase (OPH), which shows extensive substrate specificity and high hydrolytic activity that is resistant to organophosphates.

In this study, a potent MCP degrader was isolated through repetitive enrichment and successive subculturing from agricultural fields that had been subjected to long-term OP treatment. Strain YW16 can degrade not only MCP but also other related OPs. We cloned, sequenced, and expressed the OPH-encoding gene. It shares 99% identity with the *opdA* gene. This work provides the first report of this gene in the genus *Sphingobium*.

Materials and methods

Chemicals and media

All OPs (> 98% purity) were purchased from the Shanghai Pesticide Research Institute (Shanghai, China). *N*-Methylacetoacetamide (MMAA) and dimethylphosphate (> 98% purity) were acquired from Shanghai J&K Scientific Ltd. Luria-Bertani (LB) medium contained the following components (g L^{-1}): yeast extract, 5.0; tryptone, 10.0; and NaCl, 10.0; pH 7.0. The components of mineral salt medium (MSM) were as follows (g L^{-1}): K_2HPO_4, 1.5; $MgSO_4 \cdot 7H_2O$, 0.2; KH_2PO_4, 0.5; NaCl, 0.5; and NH_4NO_3, 1.0; pH adjusted to 7.0. MCP (0.5 mM) that had been dissolved in double-distilled water was added to the medium when necessary. Solid medium plates contained 2.0% (*w*/*v*) agar.

Bacterial strains, plasmids, and culture conditions

The pUC118 plasmid was used as a cloning vector, and pET29a was used as an expression vector. We used *E. coli* DH5α cells as the hosts of the cloning plasmids, and *E. coli* BL21(DE3) cells for the expression plasmids. Strain YW16 was grown aerobically at 30 °C. The *E. coli* strains were grown on LB medium, to which the appropriate antibiotics were added, at 37 °C.

Strain isolation and characterization

Two grams of soil was added to 90 mL of MSM that included MCP as the sole carbon source. The culture was transferred twice. The final culture (100 μL) was subsequently plated on LB agar plates for purification. One MCP-degrading isolate was chosen for further investigation. This strain was morphologically and physiologically characterized, classified, and preliminarily identified in line with the diagnostic tables for bacteria (Cowan and Steel 1965); an API 20NE and 32GN kit was also used for identification, according to the instructions of the manufacturer (bioMérieux, China). The morphological characteristics of the strains were observed using scanning electron microscopy (SEM) and light microscopy.

For the amplification of the partial 16S rRNA gene, the primers listed in Table 1 were used (Lane 1991). Amplification of the 16S rRNA gene sequence was carried out by Takara Bio. The 16S rRNA gene sequence of strain YW6 was compared to reference sequences using BLAST, and related sequences from GenBank were aligned with CLUSTAL-W. MEGA 5.0 software was employed to construct the phylogenetic tree (Tamura et al. 2011). Extraction and identification of fatty acids were performed by the Microbial Identification System (Sasser 1990). We determined the G+C content of strain YW16 by thermally denaturing a DNA sample (Zhang et al. 2012).

Degradation of MCP and MMAA by strain YW6 in culture

When strain YW16 had grown to late logarithmic phase, the cells were concentrated at 6000×*g* for 10 min and washed twice using sodium phosphate buffer (50 mM). Then, the OD_{600nm} of the cells was adjusted to 1.0, and the sample was used as an inoculant. Degradation of MCP was performed in 100 mL of MSM, as described above, containing 0.5 mM MCP. Samples were collected to determine the concentrations of MCP and MMAA and cell growth. The medium cultivated with heat-killed YW16 cells was considered as control.

Then, 1.0 g L^{-1} of glucose, sodium acetate, sodium citrate, sucrose, or maltose was added to MSM to determine the effects of other carbon sources on MCP biodegradation. The cultures were incubated at 10, 20, 25, 30, 37, and 40 °C, to study the effect of temperature on MCP metabolism. The cells were added into cultures at 1% (*v*/*v*) then cultivated at 30 °C at 180 rpm. Each treatment was performed in triplicate.

Cloning and expression of the *opdA* from strain YW16

The shotgun method was used to clone the OPH gene from strain YW16. Total DNA was acquired from strain YW16 in line with the method described by Sambrook et al. (Sambrook and Russel 2011) and partially digested with *Sau*3AI. The

fragments (3–9 kb) were isolated using a DNA Gel Extraction Kit and ligated with pUC118. The recombinant plasmid was transformed into *E. coli* DH5a, then plated on LB plates supplemented with 0.3 mM chlorpyrifos and 100 mg L^{-1} ampicillin. The LB plates were cultivated at 37 °C for 16 h and then at 10 °C for 48 h. Cells that produced transparent halos were considered positive, and the sequences inserted in the recombinant plasmids were subsequently analyzed. SEFA-PCR was used to obtain the full-length *opdA* gene cluster (Wang et al. 2007). The primers that were used to amplify the upstream fragment of *opdA* are presented in Table 1.

The *opdA*-n gene (*opdA* gene removing the predicted signal peptide-encoding sequence) was amplified using the genome of YW16. The primers that were designed based on the sequence analysis results are listed in Table 1. The *opdA*-n gene was ligated with pET29. The resulting plasmid was designated pET-opdA and was transformed into *E. coli* BL21(DE3), then cultured at 25 °C in auto-inducing ZYM-5052 medium supplement with kanamycin (50 μg mL^{-1}) (Studier 2005). The cells were subsequently collected, washed, resuspended with Tris-HCl buffer (10 mM, pH 8.0), and broken via sonication. Each purification process was carried out at 4 °C.

Enzyme assay

Enzymatic activity associated with various OPs was determined in line with the description of Li et al. (2012). The assays were carried out in sodium phosphate buffer (50 mM, pH 8.0) at 37 °C. The amount of substrate hydrolyzed during the process was below 10%. Briefly, 3 mL of preincubated enzyme solution (1–5 μg mL^{-1}) was supplied with the stock solution (1 μL), followed by incubation for 30 to 60 s, deciding by the substrate. One enzymatic activity unit was defined as the amount of enzyme that catalyzed the formation of 1 μmol of product per minute. For kinetic analyses, each substrate was diluted to at least five different concentrations around the K_m values. Values of kinetics parameters were acquired from Lineweaver-Burk plots.

Extraction of samples (pesticide residue) for analysis

Samples (5 mL) from the culture flasks were collected at 10,000 rpm for 10 min. Then the supernatant was extracted twice with one volume of dichloromethane, and the organic phase was evaporated at room temperature under nitrogen. The residues were redissolved in methanol (1 mL), then filtered using a Millipore membrane (0.22 μm). The samples were diluted before HPLC analysis when required. The MCP concentrations in the samples were determined via HPLC as described below.

A separation column (4.6 mm × 250 mm × 5 μm; ZORBAX SB-C18; Agilent) was utilized for HPLC analysis. The mobile phase contained methanol and water at 70:30 (*v*/*v*), and the rate was 1 mL per min. The injection volume was 20 μL, and the wavelength was 230 nm.

GC-MS analyses were carried out in electron ionization (EI) mode (70 eV) with a Finnigan gas chromatograph equipped with an MS detector. An RTX-5MS column (15 m × 0.25 mm × 0.25 μm, Restek Corp) was installed in the gas chromatograph. The column temperature was programmed to ramp from 50 (1.0 min hold) to 100 °C at 20 °C/min and then from 100 to 180 °C at 8 °C/min; the temperature was increased to 265 °C at 50 °C/min and then held at 260 °C for 2 min. The carrier gas was helium at a constant flow rate of 1.0 mL per min. The samples were analyzed in split mode (1:20) at an injection temperature of 220 °C and an EI source temperature of 250 °C and were detected in the mass range from 30 to 650 *m*/*z*.

Nucleotide sequence accession numbers

The GenBank accession numbers of the 16S rRNA and *opdA* genes are JQ666844 and KY368170, respectively.

Results

Isolation and characterization of an MCP-degrading bacterium

Four bacterial strains were isolated after their enrichment. The degradation performance of one bacterial strain was analyzed in detail, and the strain was designated YW16. Strain YW16 was a non-mobile, rod-shaped, Gram-negative bacterium (0.2–0.4 × 0.8–1.0 μm) (Fig. S1) and could grow on methanol, glucose, citrate, maltose, and sucrose. However, it could not grow on D-mannose, D-mannitol, L-arabinose, gluconate, *N*-acetyl-D-glucosamine, n-caprate, phenylacetate, malate, or adipate.

The 16S rRNA gene sequence of YW16 shared the highest sequence identity with those of *Sphingobium yanoikuyae* (99.6%), *Sphingobium scionense* (98.7%), and *Sphingobium amiense* (98.2%) (Fig. 1). The fatty acid profile of YW16 was compared with those in the commercial library TSBA40. Cellular fatty acid analysis revealed that summed feature 8 ($C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$) (46.0%), $C_{16:0}$ (12.4%), summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$) (18.2%), and 11-methyl $C_{18:1}\omega7c$ (2.3%) were the major non-hydroxylated fatty acids, and $C_{14:0}$ 2-OH (7.9%) was the major hydroxylated fatty acid. The fatty acid profile of YW16 shared common features of the genus *Sphingobium* (Takeuchi et al. 2001). The G+C content of strain YW16 was 62.1 mol%.

Thus, strain YW16 should be classified as a *Sphingobium* strain based on the physiological and chemotaxonomy characterization, as well as morphological data. Strain YW16 was

Table 1 PCR primers used in this study

Primer	DNA sequence (restriction enzyme sites are underlined)	Purpose
16S-27F	5′-AGAGTTTGATCMTGGCTCAG-3′	16S ribosomal gene
16S-1492R	5′-GGTTACCTTGTTACGACTT-3′	
5SP1	5′-GGACGAGGCTGAACAACCAAACGATAC-3′	Amplification of the upstream sequences of the *opdA* gene by SEFA-PCR
5SP2	5′-TCGTCGGTGAACTCGACGACGAAGC-3′	
5SP3	5′-GAAGCGAACCTCGTANNNNNNNNNATCGTG-3′	
opdA-F	5′-CTGATTGCATATGCGACCAATCGGTACAGGCGA-3′	Amplification of *opdA* gene for expression
opdA-R	5′-TACAAGCTTTCGTTCGGTATCTTGACGGGGA-3′	

DNA letter codes: N = A, T, G, or C. The underlined sequences, CATATG and AAGCTT, indicate *Nde*I and *Xho*I restriction sites, respectively

deposited in the China Center for Type Culture Collection (CCTCC 2011077).

Degradation ability of strain YW16

In MSM medium, the cell density of strain YW16 increased from 0.01 to 0.21 (OD_{600nm}) in 48 h, with a decrease in MCP (to a non-detectable level) and an increase in MMAA, indicating that YW16 can utilize MCP as the sole carbon source. When MCP disappeared, MMAA could not be degraded by strain YW16, during the entire degradation period (Fig. 2a). However, strain YW16 could use dimethylphosphate as the carbon source for growth (Fig. 2b).

When strain YW16 was grown with glucose, MCP was simultaneously co-metabolized, with 0.5 mM MCP being degraded in 24 h. Other carbon sources, such as maltose, sucrose, and sodium citrate, could also boost the degradation ability of MCP (Fig. 3a). The results indicated that the MCP degradation of YW16 was influenced by the presence of other sources of carbon.

Strain YW16 efficiently degraded MCP at temperatures between 20 and 35 °C. The optimum degradation temperature was 30 °C. When the temperature fell to 20 °C or rose to 40 °C, MCP biodegradation decreased, illustrating that lower and higher temperatures were disadvantageous to the biodegradation of MCP by strain YW16 (Fig. 3b).

Cloning and analysis of organophosphorus pesticide hydrolase gene clusters

Two positive clones, designated P1 and P2, were selected from approximately 5000 transformants. The fragments that had been inserted in their recombinant plasmids were sequenced, and the lengths of the inserts were found to be 4605 and 4588 bp, respectively. Interestingly, the two fragments exhibited an overlap region of 2979 bp, and the final

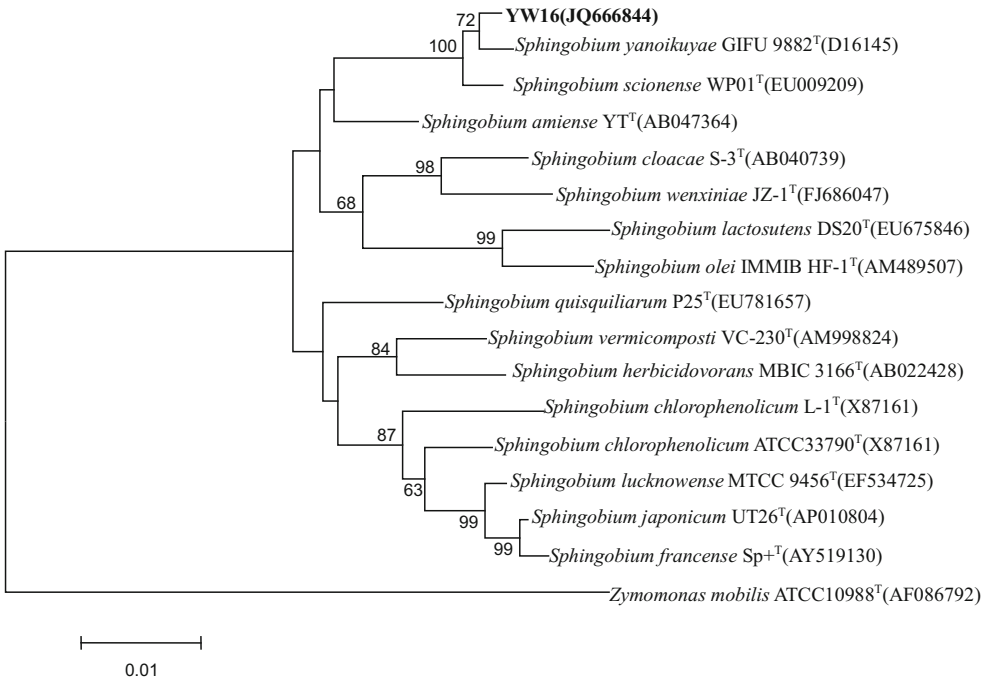


Fig. 1 Phylogenetic tree of *Sphingobium* sp. strain YW16 and related species constructed on the basis of 16S rRNA sequences using the neighbor-joining method. Bar, 0.01 Knuc unit. Bootstrap values (1200 resamplings) are shown at the branch points

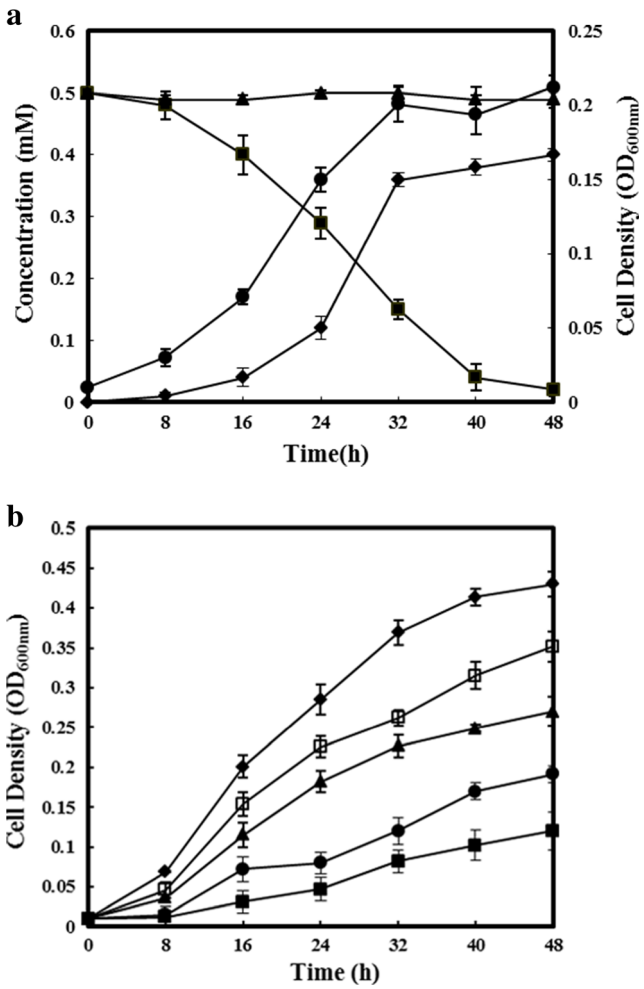


Fig. 2 **a** Utilization of monocrotophos as the sole carbon source for growth by strain YW16. (filled circle) Cell density; (filled square) concentration of monocrotophos; (filled triangle) control; and (filled diamond) concentration of MMAA. **b** Effect of the initial concentration of dimethylphosphate on the growth of strain YW16: (filled square) 0.2 mM dimethylphosphate; (filled circle) 0.5 mM dimethylphosphate; (filled triangle) 1.0 mM dimethylphosphate; (open square) 2.0 mM dimethylphosphate; and (filled diamond) 5.0 mM dimethylphosphate. The data are presented as the mean ± standard deviation for triplicate treatments

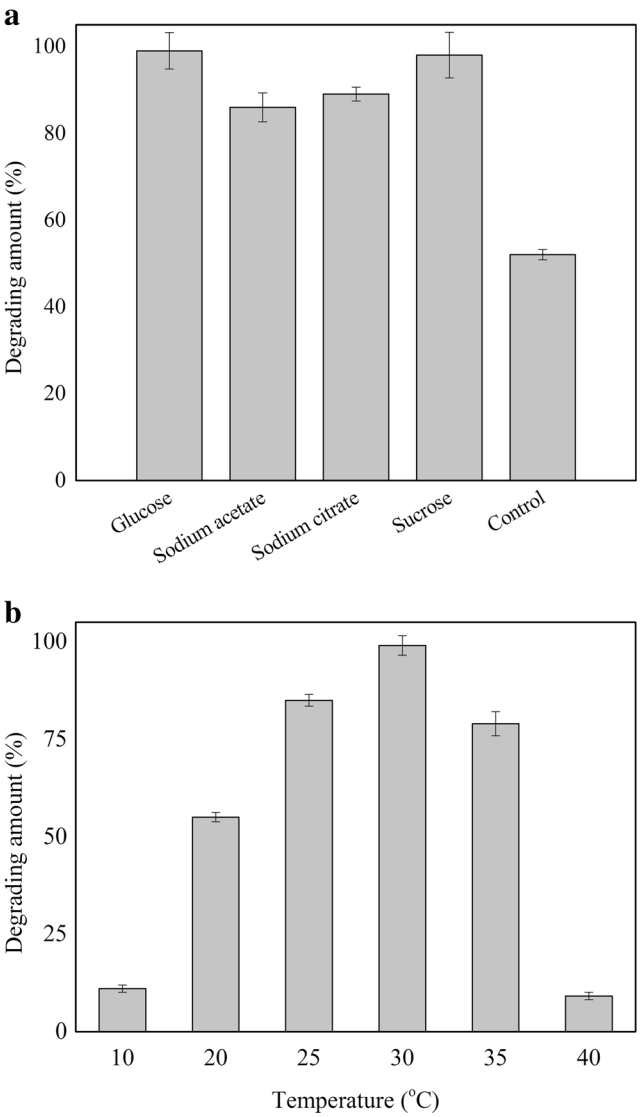


Fig. 3 **a** Effect of carbon sources on the degradation of monocrotophos by strain YW16 over 24 h. **b** Effect of temperature on monocrotophos degradation by strain YW16 in MSM over 48 h. The initial concentration of monocrotophos was 0.5 mM. The data are presented as the mean ± standard deviation for triplicate incubations

spliced fragment was 6214 bp in length. The upstream region of the *opdA* gene fragment in strain YW16 was amplified via SEFA-PCR, producing a 1041-bp fragment. The G+C content of the *opdA* cluster (7067 bp, 60.5%) was lower than that of YW16 (62.1%). Six ORFs were identified in the 7067-bp fragment, which were analyzed using the BLASTx program (www.ncbi.nlm.nih.gov). The *opdA* gene (complement of bp 3000–4154) of strain YW16 was located in the middle of the fragment, and its encoded protein, OpdA (an OPH), had a signal peptide that was 27 amino acids long. The G+C content of the *opdA* gene was 57.06%. The amino acid sequence of OpdA shared 99% similarity with that of OpdA of *Agrobacterium tumefaciens* (GenBank Accession No. AY043245) (Horne et al. 2002a). Five other ORFs were found to flank the *opdA* gene. Among these ORFs, a putative transposase (complement of bp 1–765, highest similarity of 99%, with A. *tumefaciens*); a putative integrase gene (complement of bp 887–1999, highest similarity of 95%, with *A. tumefaciens*); and a putative ATP-binding protein gene (complement of bp 1996–2853, highest similarity of 95%, with A. *tumefaciens*) were located upstream of the *opdA* gene. A putative aromatic hydrolase gene (complement of bp 4126–5091, highest similarity of 91%, with *Flavobacterium* sp. strain ATCC 27551) and a conjugal transfer protein (TraI) gene (complement of bp 5214–6689, highest similarity of 69%, with *Sphingobium* sp. SYK-6) were located downstream of the *opdA* gene (Fig. 4).

E. coli BL21(DE3) was used to express the *opdA* gene, and SDS-PAGE was employed to purify the product. The molecular weight of the target protein was approximately 35 kDa, indicating that the signal peptide had been cleaved (Fig. 5). The activity of OpdA was determined by measuring the enzymatic turnover of MCP in its presence.

GC-MS was used to identify the metabolites. Product A, with characteristic second-order MS-fragment ion peaks at 223, 192, 127, 144.48, 109, 97, 67, and 58 *m*/*z*, was identified as MCP; one degradation product (designated product B) appeared during the biodegradation of MCP in PBS buffer; its characteristic second-order MS-fragment ion peaks were located at 115, 87, 73, and 58 *m*/*z*, and its molecular weight was estimated to be 115 (Fig. S2). Using these results and comparing sample retention times and mass spectrum patterns of standards, we preliminarily identified product B as *N*-methylacetoacetamide, which illustrates that the degradation of MCP by OpdA is due to the hydrolysis of the phosphotriester bond (Fig. S3).

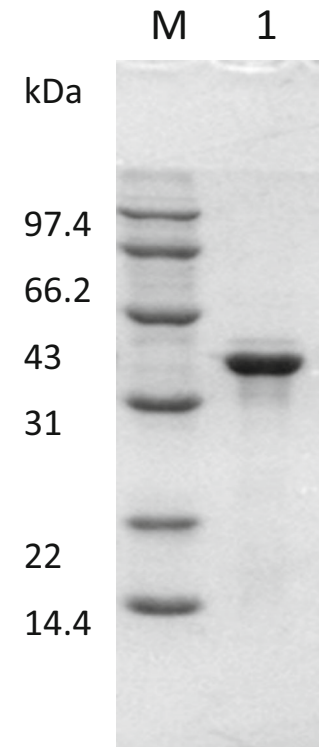


Fig. 5 SDS-PAGE of purified OpdA-His_6. Lane M, molecular mass standards (molecular masses are indicated on the left in kDa); lane 1, purified OpdA-His_6

Kinetic analysis of the enzyme

All of the tested OpdA substrates (except for trichlorfon, which exhibits a P-C bond) harbored a P-O-Z moiety (where Z stands for the leaving group) and either a thion (P=S) or an oxon (P=O). OpdA was able to hydrolyze OPs that were tested, with the hydrolysis rates decreasing as follows: methyl parathion > fenitrothion > phoxim > dichlorvos > ethyl parathion > trichlorfon > triazophos > chlorpyrifos > MCP > diazinon (Table 2). Of these substrates, methyl parathion was the most preferred substrate. The aryl or heterocyclic group in the leaving group may serve a vital function in the stereochemical and steric selection of substrates by OpdA. For diazinon and MCP, which are OPs with a P-O-Z moiety, the catalytic efficiency (k_{cat}/K_m) was only 0.069 and 0.070, respectively. Comparing the structures of the substrates indicated that the existence of an isopropyl group in the heterocycle greatly hindered the enzyme-substrate interaction. The low catalytic efficiency of MCP may be due to the lack of an aryl or heterocyclic group.

Discussion

OPs are generally stable at faintly acidic to neutral pH values and decompose readily in solutions with an alkaline pH, with the exception of diazinon, which is unstable at acidic pH values (Singh et al. 2003a, b). Environmental degradation rates of MCP have been extensively studied. Soil degradation includes chemical hydrolysis and microbial activity (Beynon et al. 1973). The high rate of MCP degradation in alkaline soil initially owed to chemical degradation. In recent years, several studies have found that the rate of MCP degradation is low in soils with an acidic pH, while the degradation of MCP substantially increases at higher soil pH values (Lee et al. 1990).

The recognition that organisms in the soil can affect ecosystem processes has led to increased research. Microorganisms

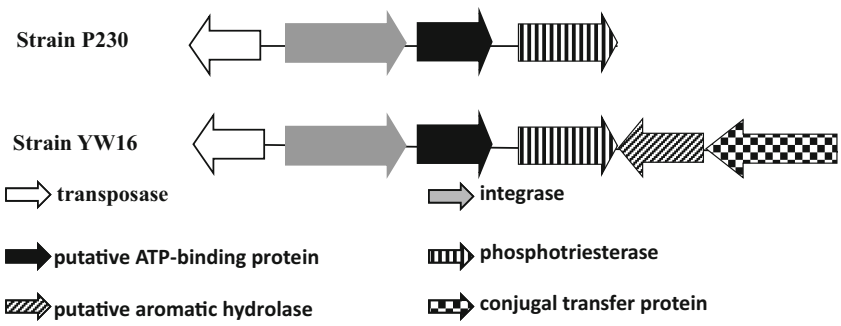
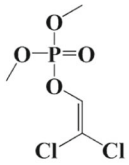
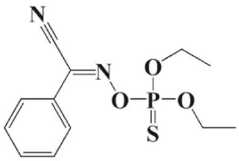
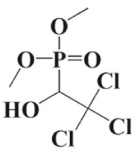
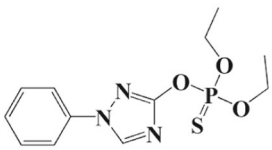
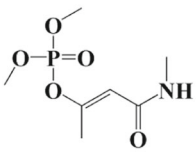
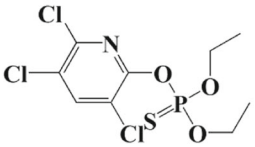
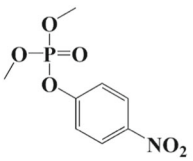
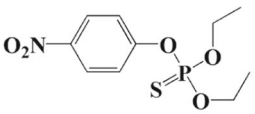
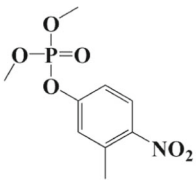
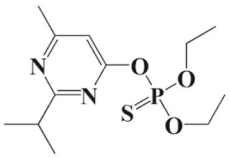


Fig. 4 Comparison of *opdA* gene clusters of strain YW16 and strain P230; the coding regions and transcriptional directions of the genes are indicated with broad arrows

Table 2 Substrate specificity and apparent kinetic constant of OpdA

OPs/Structures	K_m (μM)	k_{cat} (min^{-1})	OPs/Structures	K_m (μM)	k_{cat} (min^{-1})
Dichlorvos 	71.6±2.3	20.1±0.8	**Phoxim** 	99.8±3.1	29.6±2.5
Trichlorfon 	80.6±5.9	15.2±1.9	**Triazophos** 	140.7±3.8	30.5±1.3
Monocrotophos 	117.2±1.9	8.2±1.2	**Chlorpyrifos** 	126.4±7.6	17.5±2.5
Methylparathion 	65.3±4.3	42.7±4.6	**Ethylparathion** 	108.5±8.1	30±3.1
Fenitrothion 	80.5±1.7	31.3±1.2	**Diazinon** 	198.1±9.3	13.7±2.1

Values are means ± standard deviations from triplicate measurements

are reported to be the main agents that remove OPs in soil (Singh and Walker 2006). *Sphingobium* species are major catabolic "players" due to their ability to degrade various pollutants. They are noted for their metabolic multifunctionality and active role in aerobic degradation (Wang et al. 2014; Wittich et al. 2007; Yan et al. 2010; Zhang et al. 2012). Strains from the *Sphingobium* genus that are able to degrade OPs have been previously isolated (Harper et al. 1988). In this report, we isolated a novel MCP-degrading strain, *Sphingobium* sp. YW16, that could degrade over 95% of MCP when present at a concentration of 0.5 mM in 48 h (Fig. 2a).

Strain YW16 first hydrolyzed MCP into dimethylphosphate and MMAA. MMAA accumulated during the degradation of MCP and has been reported to be a degradation product of MCP in soil and pure cultures (Beynon et al. 1973; Bhadbhade et al. 2002a, b; Gundi and Reddy 2006). However, dimethylphosphate was not detected via HPLC; dimethylphosphate may have avoided detection because it exhibits no UV absorbance or because it could be utilized by YW16 as a carbon source to support its growth (Fig. 2b). Strain YW16 was capable of using dimethylphosphate as its sole carbon source, meaning that it could use the methanol released from the breakdown of the phosphodiester bond. This characteristic of YW16 could represent an advantage in obtaining nutrients, especially in nutrient-poor environments.

Strain YW16 was also capable of hydrolyzing other OP insecticides, including dichlorvos, trichlorfon, MCP, methyl parathion, fenitrothion, phoxim, triazophos, chlorpyrifos, ethyl parathion, and diazinon. These compounds exhibit diethyl (or dimethyl) phosphorothionate side chains and will also

release dimethyl and diethyl compounds similar to that of MCP and could therefore be employed by strain YW16 as its carbon source. Thus, strain YW16 presents enormous potential for the bioremediation of OP-contaminated sites.

The common initial step in the metabolism of organophosphates is the hydrolysis of the phosphoester bond, which is catalyzed by OPH (Cui et al. 2001; Harper et al. 1988; Horne et al. 2002a, b; Shen et al. 2010). Several OPH-encoding genes, including *opd*/*opdA*, *mpd*, *ophC2*, *ophB*, and *opaA*, have been reported (Singh 2009); among these genes, *opd*/*opdA* and *mpd* have been extensively studied. Many strains that carry almost identical *opd* or *mpd* sequences have been reported. The *opd* and *mpd* genes have been isolated from geographically, temporally, and biologically different species (Harper et al. 1988; Horne et al. 2002a; Mulbry et al. 1986; Siddavattam et al. 2003). *opdA* was first cloned from *Agrobacterium radiobacter* (Horne et al. 2002a) and is 88% identical to *opd*. OpdA exhibits approximately 10-fold higher activity than OPH against dimethyl OPs (Horne et al. 2002a). In the present study, the *opdA* gene of YW16 was cloned and expressed, providing the second report of an *opdA* gene; notably, this is the first report of this gene from *Sphingobium*.

OpdA can degrade a variety of OPs, with the hydrolysis efficiency depending on the molecular structure of the OP. The catalytic efficiency of the enzyme for methyl parathion was much higher than that for other OPs, indicating that the additional steric centre resulted in a slower hydrolysis rate owing to steric hindrance of hydrolysis or stabilization of the phosphotriester bond. The aryl or heterocyclic moiety in the leaving group may perform a vital function in the stereochemical and steric selection of substrates by OpdA.

Horizontal gene transfer (HGT) performs a vital function in the degradation of xenobiotics, including pesticides. Generally, it is associated with mobile genetic elements (MGEs) or transposons. Zhang et al. first found that the *mpd* gene clusters, including *mpd*, *tnpA* (IS *6100*), and three other ORFs, were highly conserved in seven different strains, but they did not demonstrate that transfer events occurred (Zhang et al. 2006). Wei et al. subsequently identified a similar cluster in *Pseudomonas* sp. WBC-3, which confirmed the transposability of the *mpd* gene cluster (Wei et al. 2009). *opdA* of *Agrobacterium radiobacter* is located in an insertion derivative (Tn*opdA*) in which the transposed gene and inverted repeated sequences are the same as those of IS *6100*; its transposability has also been confirmed. The *opd* of *Flavobacterium* sp. ATCC 27551 is flanked by ISFl*sp1* (a member of the IS*21* family) and Tn*3*-like element, but no transposition events were identified.

In the present study, a fragment including *opdA* was cloned from *Sphingobium* sp. strain YW16, and the average G+C content of its chromosome was found to be 62.1%. The G+C content of the *opdA* gene cluster was 60.5%, which was lower than that of its host chromosome (62.1%), providing evidence that the insert was obtained through HGT. The transposase, integrase, ATP-binding protein, and OpdA in the *opdA* gene cluster share high amino acid sequence identity (> 95%) with enzymes from *Agrobacterium radiobacter*. Interestingly, a putative aromatic hydrolase gene located downstream of the *opdA* gene was observed to be most similar (91%) to a gene of *Flavobacterium* sp. strain ATCC 27551 (Siddavattam et al. 2003). The *tra* sequences adjacent to the putative aromatic hydrolase gene show similarity (69%) to the *tra* gene of *Sphingobium* sp. SYK-6 (Fig. 4). The obtained sequence information demonstrated the presence of a *traI* gene, providing evidence of horizontal mobility of the putative aromatic hydrolase gene. There are two HGT elements in the *opdA* gene cluster: Tn*opdA*, which was acquired from *A. radiobacter* P230, and a putative aromatic hydrolase gene, which was obtained from *Flavobacterium* sp. strain ATCC 27551. Strain YW6 may have acquired the *opd*-like gene cluster that enables it to utilize OPs via HGT from a phylogenetically distinct species, which suggests that horizontal transfer of *opd*-like gene clusters may perform a vital function in the adaptation of bacterial populations to OP-polluted sites. Considering these data, the presence of the *opdA* gene cluster in strain YW16 provides further evidence that the transposition of *opdA* genes shares common features with the horizontal transfer of *opd* genes, which promotes bacterial adaptation to OP-polluted ecosystems. In addition, the horizontal transfer of *opd* genes into an autochthonal microorganism could enhance the OP-degrading strain bio-augmentation efficiency in situ bioremediation of OP-polluted sites.

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