

Cell surface display of organophosphorus hydrolase for sensitive spectrophotometric detection of *p*-nitrophenol substituted organophosphates



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ABSTRACT

Organophosphates (OPs) widely exist in ecosystem as toxic substances, for which sensitive and rapid analytical methods are highly requested. In the present work, by using N-terminal of ice nucleation protein (INP) as anchoring motif, a genetically engineered *Escherichia coli* (*E. coli*) strain surface displayed mutant organophosphorus hydrolase (OPH) (S5) with improved enzyme activity was successfully constructed. The surface location of INP-OPH fusion was confirmed by SDS-PAGE analysis and enzyme activity assays. The OPH-displayed bacteria facilitate the hydrolysis of *p*-nitrophenol (PNP) substituted organophosphates to generate PNP, which can be detected spectrometrically at 410 nm. Over 90% of the recombinant protein present on the surface of microbes demonstrated enhanced enzyme activity and long-term stability. The OPH activity of whole cells was 2.16 U/OD₆₀₀ using paraoxon as its substrate, which is the highest value reported so far. The optimal temperature for OPH activity was around 55 °C, and suspended cultures retained almost 100% of its activity over a period of one month at room temperature, exhibiting the better stability than free OPH. The recombinant *E. coli* strain could be employed as a whole-cell biocatalyst for detecting PNP substituted OPs at wider ranges and lower detection limits. Specifically, the linear ranges of the calibration curves were 0.5–150 μM paraoxon, 1–200 μM parathion and 2.5–200 μM methyl parathion, and limits of detection were 0.2 μM, 0.4 μM and 1 μM for paraoxon, parathion and methyl parathion, respectively (S/N = 3). These results indicate that the engineered OPH strain is a promising multifunctional bacterium that could be used for further large-scale industrial and environmental applications.

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1. Introduction

Organophosphorus hydrolase (EC 3.1.8.1, OPH) which was initially isolated from *Pseudomonas diminuta* MG and *Flavobacterium* sp. in 1980s [1,2], is capable of hydrolyzing a broad range of organophosphorus esters bonds such as P–O, P–S, P–F and P–CN bonds. It has been exploited as the key element for biodegradation and biosensing of organophosphate pesticides [3–5]. OPH can catalyze the hydrolysis reaction of *p*-nitrophenol (PNP) substituted organophosphorus compounds (OPs) such as paraoxon, parathion

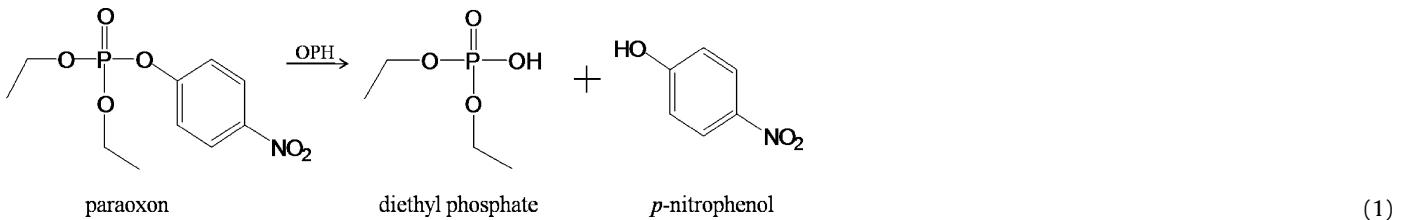
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and methyl parathion to generate phosphate esters and PNP. The typical hydrolysis of paraoxon by OPH is shown in Eq. (1).



OPs are widely used as pesticides and warfare agents in agriculture, industry and military affairs all over the world [6–8]. Additionally, OPs are generally strong neurotoxic, cholinesterase-inhibiting molecules that inflict severe toxic effects on various living organisms [9]. Organophosphorus compounds such as paraoxon, methyl parathion and dichlorvos can cause severe damage to ecosystem, which have been forbidden to produce and use. However, OPs can extensively disperse in environment for a long time and turn into persistent organic pollutants due to its stable chemical properties. Therefore, a rapid and sensitive method to detect OPs is highly desirable. Conventional detection methods mainly include gas chromatography and liquid chromatography [10–13], which require expensive instrumentation, complicated sampling handling procedures, and are therefore time-consuming. In recent years, various biosensors were developed based on enzyme and microorganism for convenient, rapid and accurate determination of OPs [4,14–18]. OPH-based biosensor with purified enzyme has been proposed to analyze various OPs [14,15]. Although the method is simple, rapid and direct, the tedious process of purifying enzymes has limited its practical applications. The transport barrier of organophosphate pesticides across the cell membrane would hinder the interaction between substrate and intracellular enzymes. Microbial surface display provides an alternative solution to purified enzyme for sensitive and cost-effective OPs detection. So far, OPH has been displayed on the surface of several microbes using different anchoring motifs, including ice nucleation protein (INP) from *Pseudomonas syringae* [19,20], Lpp-Omp A from *Escherichia coli* (*E. coli*) [21] and α -agglutinin anchor from *Saccharomyces cerevisiae* [22]. The majority of OPH-display systems have been used for detoxification of organophosphorus pesticides [11,23,24]. Unfortunately, the level of whole cell OPH activity was unsatisfactory, and thereafter, hard to meet the demands of practical applications in the fields [25]. On the other hand, a few microbial sensors with surface displayed OPH have been reported for detection of paraoxon, methyl parathion and other OPs [17,26,27]. For example, *E. coli* cells surface displayed OPH were integrated with optical transducer resulting in a microbial biosensor with detection limits of 3 μ M for paraoxon and parathion [18]. Carbon paste electrodes modified with surface-anchored OPH showed a low detection limit of 0.2 μ M of paraoxon and 1 μ M of parathion [16]. However, the above biosensors were insensitive, which is mostly probably originating from the lower level of whole cell OPH activity. The analytical performance of these biosensors could be improved by enhancing the catalytic efficiency of OPH. Nevertheless, to date, little efforts have been devoted to raise the functional expression level and enzyme activity. In the context, Roodveldt obtained an OPH variant S5 containing three point mutations that led to a 50-fold increase in esterase activity [28], which could make the strain an extremely promising candidate for the preparation of OPH biosensor. In our previous work, on basis of the cell surface display system using the N-terminal domain of INP from *P. borealis* as anchoring motif, we have successfully constructed both xylose dehydrogenase- and glucose dehydrogenase-displaying strains, by which a series of biosensors have been developed for the sensitive and selective measurement

of D-xylose [29,30] or D-glucose [31,32] and xylose-based biofuel cell was assembled [33]. In the present study, mutated OPH (S5) was displayed on the surface of *E. coli* using INP display system. The enzyme activity and stability were also investigated. And finally, the novel whole cell biocatalyst was utilized for establishing sensitive and rapid detection method for PNP substituted OPs. To the best knowledge of the authors, this is the first report on the functional surface displaying of mutated OPH for OPs detection.

2. Materials and methods

2.1. Bacterial strains and materials

E. coli DH5 α (F $^-\varphi$ 80 lacZ Δ M15 Δ (lacZYA-argF) U169 endA1 recA1 hsdR17 (r $_k^-$, m $_k^+$) supE44 λ -thi-1 gyrA96 relA1phoA) was used for constructing recombinant plasmids. *E. coli* strain BL21 (DE3) (F $^-\varphi$ ompT hsdS $_B$ (r $_B^-$ m $_B^+$) gal dcm(DE3)) was used as the host cell for the expression of recombinant protein. Cells bearing recombinant plasmid were grown in LB media with 50 mg/L kanamycin at 37 °C. Bacteria harboring expression vectors were grown to an OD of 0.6 before induction with isopropyl- β -D-thiogalactoside (IPTG) at final concentrations ranging from 0.1 mM to 1 mM for 8 h at 25 °C. INP was generously provided by Prof. Virginia K. Walker (Department of Microbiology and Immunology, Queen's University, Canada). Buffer solutions with different pH values were prepared: 20 mM citrate-NaOH buffer, pH 3–5.5; 20 mM phosphate buffer, pH 5.5–7.5; 20 mM Tris-HCl buffer, pH 7.5–8.5; 20 mM glycine-NaOH buffer, pH 8.5–10.5.

2.2. Construction of INP-OPH fusion

The *inaPb-opp* fusion was constructed as follows. The OPH-coding *opp* gene originally from *P. diminuta* (without leader peptide-coding gene) was amplified from plasmid pMAL-c2x/S5, which was a kind gift from Dr. Roodveldt (Department of Biological Chemistry, the Weizmann Institute of Science, Israel). Forward (5'-CGCGGATCCATACCAACAGCGCGATCGG-3') and reverse (5'-CCCAAGCTTGACGCCGCAAGGTCGGTGA-3') primers were applied to introduce *Bam*H I and *Hind*III restriction sites (underlined). The amplified fragment was ligated to pGEM-T easy vector and digested with *Hind*III and *Bam*H I, and then inserted into the same sites of vector pTInaPb-N, resulting in plasmid pTInaPb-N/Oph. The plasmid pTInaPb-N was the ligation of *inaPb* and pET-28a (+) conserved in our lab. Finally, the recombinant plasmid pTInaPb-N/Oph was transformed into *E. coli* BL21 (DE3) and expressed under the control of T7 promoter.

2.3. Whole cell OPH activity assay

The OPH activity assay was based on a method developed by Shimazu et al. [19]. *E. coli* cells harboring plasmid pTInaPb-N/Oph were grown for 8 h after IPTG induction, harvested, and resuspended in 75 mM Tris-HCl buffer with 50 μ M CoCl₂ (pH 8.0). For each assay, 200 μ L of cells (OD₆₀₀ = 1.0) were added to 700 μ L of Tris-HCl buffer and 100 μ L of 20 mM paraoxon in 20% methanol. The reaction was conducted in 1.5 mL plastic tube for 2 min at 37 °C and then centrifuged at 12,000 rpm for 1 min to remove the cells. 200 μ L supernatant was added in a 96-deep-well plate and the production of PNP was determined by measuring the absorbance at 410 nm where PNP absorbs strongly at ambient temperature using microplate reader. Enzyme activities were expressed as μ mol of paraoxon hydrolyzed per minute (U) per OD₆₀₀ whole cells.

2.4. Cell fractionation and SDS-PAGE analysis

Cells harboring INP-OPH fusion were harvested and resuspended in 15 mL Tris-HCl buffer (pH 8.0) containing 1 mM EDTA and 10 μ g/ml lysozyme. Then the cells were incubated on ice for 1 h. The cells were disrupted by ultrasound sonication. After centrifugation at 6000 rpm for 10 min, the cell debris was removed and

the supernatant was total cell protein. To obtain membrane fraction, the supernatant was centrifuged at 39,000 rpm for 1 h with an ultracentrifuge. The pellet (total membrane fraction) was resuspended with PBS buffer containing 0.01 mM MgCl₂ and 2% Triton X-100 and was then incubated for 30 min at room temperature for solubilizing the inner membrane (IM). Subsequently, the outer membrane (OM) fraction was repelleted after centrifugation at 39,000 rpm for 1 h. All components were suspended in Tris-HCl buffer (pH 8.0) for further analysis. SDS-PAGE was conducted to determine the location of OPH expression. Cytoplasm and membrane fractions of *E. coli* BL21 harboring pET-28a (+) were prepared as a control.

2.5. The effect of temperature and pH on OPH activity

E. coli cells harboring plasmid pTInaPb-N/Oph were grown in LB medium for 8 h after IPTG induction, harvested, and resuspended in 75 mM Tris-HCl buffer (pH 8.0) containing 50 μM CoCl₂. To determine the optimal temperature of OPH activity, the enzyme activity assay was conducted under different temperatures (20–80 °C) in Tris-HCl buffer (pH 8.0). To investigate the pH stability of cell surface displayed OPH, equal volumes of cells were incubated at 4 °C in different buffer at a pH between 3 and 10.5 for 1 h. The residual activity was determined under standard condition.

2.6. Stability of culture expressing INP-OPH fusion protein

OPH surface-displaying bacteria cells were suspended in 75 mM Tris-HCl buffer with 50 μM CoCl₂ (pH 8.0) and incubated at different temperatures. The same amount of sample solution was taken out for OPH activity assay at regular intervals for a month.

2.7. Substrate specificity assay

Substrate specificity of surface displayed OPH was determined by measuring the enzyme activity toward various organophosphorus pesticides, including parathion, methyl parathion, fenitrothion, phoxim, chlorpyrifos and dimethoate. All reactions were conducted in Tris-HCl buffer solution (pH 8.0) containing 1 mM substrate and 50 μM CoCl₂ at 37 °C for 2 min. Hydrolysis of organophosphorus pesticides except fenitrothion was measured spectrophotometrically by monitoring the production of *p*-nitrophenol at 410 nm. Hydrolysis of fenitrothion was detected at 358 nm spectrophotometrically by quantifying the resultant 3-methyl-4-nitrophenol.

2.8. Detection of PNP substituted OPs using cell surface displayed OPH

The detection of PNP substituted OPs was conducted in Tris-HCl buffer (75 mM, pH 8.0) containing 50 mM CoCl₂, recombinant cells (200 μl, OD₆₀₀ = 1) and varying concentrations of paraoxon (0–250 μM), parathion (0–250 μM) and methyl parathion (0–250 μM) at 37 °C for 10 min. After removing the cells, absorbance at 410 nm was measured. All experiments were repeated at least three times.

3. Results and discussion

3.1. Surface expression of OPH using INP

In an attempt to target OPH to the surface of *E. coli*, a truncated INP was used as the anchoring motif, which has been used to efficiently target enzymes to the surface of *Moraxella* sp., *Salmonella* spp., *P. putida* and *E. coli* with no influence on cell viability [19,34,35]. Plasmid pTInaPb-N/Oph with *inaPb-opd* fusion was constructed and transformed into *E. coli* strain BL21 (DE3) to express INP-OPH. Strain harboring vector pTInaPb-N/Oph was grown at 25 °C and induced with IPTG to produce fusion protein. Cells were disrupted by sonication after induction, and then the fraction of inner membrane and outer membrane were separated. To investigate whether OPH was properly expressed on the surface of *E. coli*, SDS-PAGE was conducted to analyze protein expression of different cellular fractions. As shown in Fig. 1, a particular band corresponding to INP-OPH at 55 kDa was detected in total cell lysate and outer membrane fraction of *E. coli* cells harboring pTInaPb-N/Oph, while there was no such band detected in inner membrane fraction, cytoplasmic fraction and all fractions of negative control strains. So it is confirmed that INP-OPH fusion was correctly displayed on the surface of *E. coli* strain BL21 in a steady conformation.

3.2. Whole-cell OPH activity of surface displayed OPH

E. coli harboring pTInaPb-N/Oph was cultured in LB medium until OD₆₀₀ reached about 0.6, and IPTG was added to a final

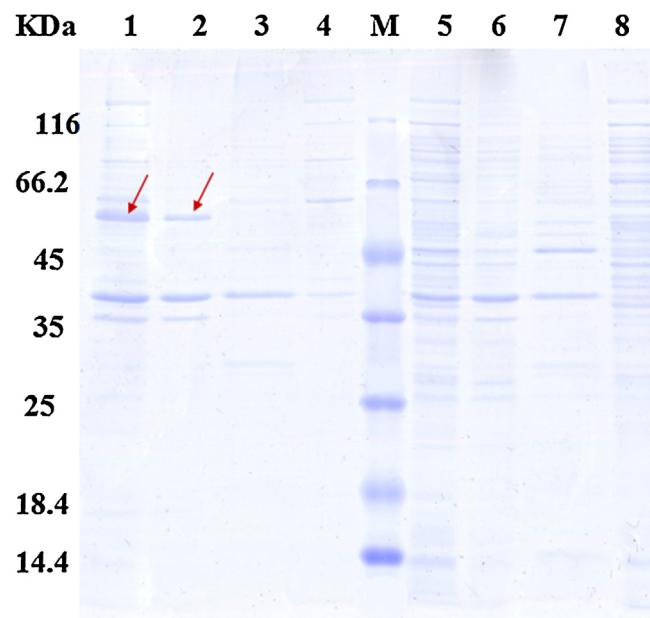


Fig. 1. SDS-PAGE analysis of INP-OPH fusion in different cellular fractions. Lane M, protein standard markers. Lanes 1–4, *E. coli* cells harboring pTInaPb-N/Oph: lane 1, total cell lysate lane 2, outer membrane fraction; lane 3, inner membrane fraction; lane 4, cytoplasmic fraction. Lanes 5–8, *E. coli* cells harboring pET28a (+): lane 5, total cell lysate; lane 6, outer membrane fraction; lane 7, inner membrane fraction; and lane 8, cytoplasmic fraction.

concentration of 0.1 mM, 0.2 mM, 0.5 mM and 1 mM, respectively. After an 8 h induction at 25 °C, the cells were harvested and washed twice with Tris-HCl buffer (pH 8.0). The enzyme assay was conducted using paraoxon as its substrate. The maximum whole cell OPH activity was obtained when the cells were incubated with 0.1 mM IPTG (data not shown). The further increase in concentration of IPTG led to the decline of catalytic efficiency due to the formation of inclusion body and growth inhibition at speeding transcription rates. As shown in Table 1, the OPH activity of whole cells was 2.16 U/OD₆₀₀ (12.44 U/mg cells), and over 90% of the total activity was found in the outer membrane fraction, which is in accordance with the distribution of INP-OPH recombinant protein on SDS-PAGE gels. The intracellular expression of mutated OPH (S5) was investigated in previous study, which was obtained by directed evolution to improve enzyme activity [28]. The esterase activity of OPH mutant was 50-fold higher than the wild type. In our study, the OPH mutant was fused with INP for surface display on bacteria, and the recombinant protein exhibited significant paraoxonase activity which was much higher than those observed in several previously reported OPH-displaying systems. For example, *E. coli* strain MB109-406 exhibited a whole cell OPH activity of 0.62 U/mg cells [36], which was just 5% of our OPH activity. Yang reported that the activity of cell-surface-displayed-OPH was 0.039 U/OD₆₀₀ [25], which was only 1.8% of our data. Thus, our results indicated that OPH was successfully displayed on the surface of *E. coli* strain BL21 (DE3) with excellent OPH activity.

3.3. The effects of temperature and pH on OPH activity

The effects of temperature and pH on the enzymatic activity of surface displayed OPH were investigated. As shown in Fig. 2A, the optimal temperature for OPH activity was around 55 °C, which increased 20 °C in comparison with other purified OPH [37]. However, enzymatic activity dropped by 82% at 20 °C and decreased dramatically at temperature above 70 °C.

Table 1

OPH activity of different subcellular fraction of *E. coli* harboring expression vectors.

OPH activity (U/OD ₆₀₀)					
Plasmid	Entire cell	Cell lysate	Outer membrane	Cytoplasm	Inner memberane
pTInaPbN-Oph	2.16 ± 0.05	1.96 ± 0.08	1.87 ± 0.11	0.09 ± 0.002	0.13 ± 0.004
pET28a(+)	ND	ND	ND	ND	ND

The activity was measured with paraoxon as the substrate. Activities were expressed in units (1 μmol of substrate hydrolyzed per min) per OD₆₀₀ whole cells. ND, not detectable. Data are shown as mean value ± standard deviations from three separate experiments.

The whole cell could retain over 50% of its initial activity within pH 6.5–9 (Fig. 2B). After incubation for 1 h at pH 3–6, less than 10% of OPH's initial activity remained. The sharp decline of OPH activity below pH 7 was probably due to the conformation disruption of its active site. The effect of pH on surface displayed OPH activity was in fairly agreement with free enzyme in a previous study [37].

3.4. Stability of cell surface displayed OPH

In order to test the stability of INP-OPH fusion protein, the whole cell was incubated in Tris-HCl buffer (pH 8.0) at different temperatures and the OPH activity was determined intervally. As shown in

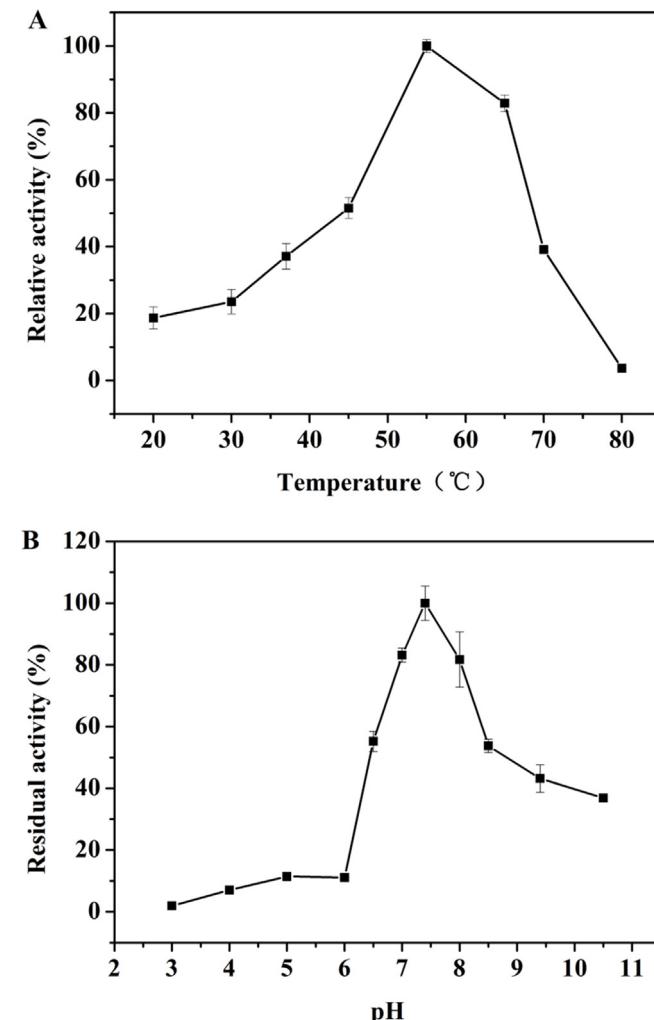


Fig. 2. (A) The effect of temperature on OPH activity. Enzyme activities were measured at different temperatures in standard reaction system. (B) The effect of pH on OPH activity. Residual activities were determined after incubating in buffer solutions of various pH values for 1 h. Absorbance at 410 nm was determined using a microplate reader. The results were obtained in three independent experiments, and the error bars are shown.

Fig. 3A, the entire cell activity of *E. coli* remained essentially the original level over one month period at ambient temperature, which showed great potential in biosensor and biodegradation application. When the temperature increased to 40 °C, 52% OPH activity was lost after one month incubation (Fig. 3B). The half-life of the whole cell was 2.5 days at 60 °C (Fig. 3C). These results demonstrated that the surface display of OPH using N-terminal domain of INP as anchoring motif did not disturb the membrane structure, which is in accordance with other INP surface-display systems [19,38]. The OPH variant S5 lost its all paraoxonase activity when the crude enzyme was incubated above 60 °C for 20 min [28]. In our present study, cell surface displayed mutant OPH showed higher stability than free protein probably owing to the INP-based cell surface display system.

3.5. Substrate specificity

Substrate specificity of *E. coli* displayed OPH was examined. PNP substituted OPs can be hydrolyzed to generate PNP, which can be monitored by spectrometric method. The relative hydrolytic OPH activities of the whole cell toward parathion, methyl parathion, fenitrothion, phoxim, chlorpyrifos and dimethoate are listed in Table 2. Obvious absorbances at 410 nm were observed when parathion and methyl parathion were added to the reaction system. However, very little signal was detected for fenitrothion, and no absorbance peak was observed when other three OPs without binding PNP were present. These results indicated that only PNP substituted OPs could be detectable by our established approach, which meant no interference from other OPs with regard to the determination of PNP substituted OPs.

3.6. Detection of PNP substituted OPs using cell surface displayed OPH

Based on the established conditions earlier, absorbances at 410 nm for different concentrations of paraoxon (0–250 μM) in Tris-HCl buffer (75 mM, pH 8.0) containing 50 mM CoCl₂ and recombinant cells (200 μl, OD₆₀₀ = 1) were measured using microplate reader. The absorbance at 410 nm as a function of paraoxon concentration is shown in Fig. 4, from which the absorbance was linear with paraoxon concentration within 0.5–150 μM. Thereafter, the absorbance response was leveled off

Table 2

Substrate specificity of surface displayed OPH.

Substrate	Absorbance at 410 nm	Relative activity (%)
Paraoxon	1.459 ± 0.015	100
Parathion	0.801 ± 0.007	54.83
Methyl parathion	0.331 ± 0.008	22.69
Fenitrothion	0.077 ± 0.004	5.28
Phoxim	0	0
Chlorpyrifos	0	0
Dimethoate	0	0

The OPs-hydrolyzing activity was assayed with seven OP pesticides as the substrate that described in Section 2. Data are shown as mean value ± standard deviations from three separate experiments.

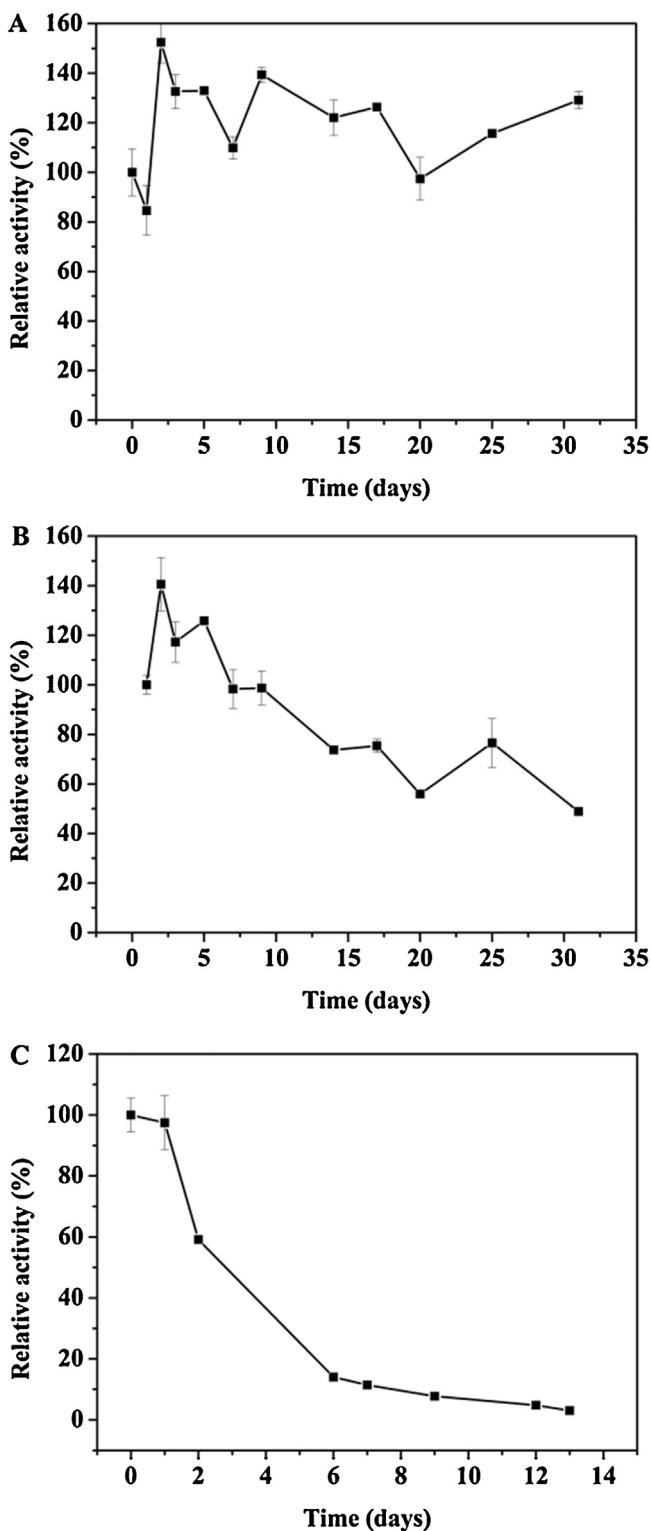


Fig. 3. Stability of cell surface displayed OPH varying incubation temperature. (A) Room temperature; (B) 40 °C; and (C) 60 °C. Residual activities were measured after incubating for various times at different temperature. Enzyme activity assays were conducted in standard condition. The results were obtained in three independent experiments, and the error bars are shown.

upon further increase of paraoxon concentration, suggesting that it is feasible to detect paraoxon based on cell surface display system using spectrophotometric method. The limit of detection (LOD), which is defined as three times the standard deviation of the response obtained for a blank ($S/N=3$), was 0.2 μM for paraoxon. Similarly, parathion and methyl parathion could also be detected

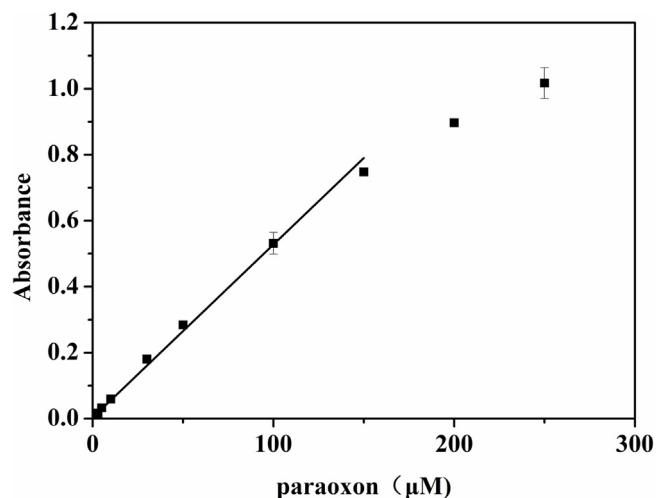


Fig. 4. Calibration curve for detection of paraoxon using cell surface displayed OPH.

Table 3

Performance of various biosensors for the detection of PNP substituted OPs.

Transducer type	Substrate	Linear range (μM)	LOD (μM)	Ref.
Optical	Paraoxon	Up to 600	3	[18]
	Parathion	Up to 30	3	
Amperometric	Paraoxon	Up to 40	0.2	[16]
	Methyl parathion	Up to 175	1	
Amperometric	Paraoxon	Up to 4	0.15	[15]
	Methyl parathion	Up to 2	0.8	
Amperometric	Paraoxon	0.2–8	0.12	[14]
Spectrophotometric	Paraoxon	0.5–150	0.2	This work
	Parathion	1–200	0.4	
	Methyl parathion	2.5–200	1	

using OPH-displayed cell, for which the linear range was 1–200 μM for parathion and 2.5–200 μM for methyl parathion. The LODs were evaluated to be 0.4 μM and 1 μM for parathion and methyl parathion, respectively. In the context, different methods were reported for determination of OPs, and the analytical performances are summarized together with our results in Table 3. The linear ranges for most methods reported so far were unclear, only the upper limits of detection were shown [4,16,27]. Moreover, the LOD value of paraoxon with our approach was 15-fold lower than the fiber optic microbial biosensor [18]. More importantly, our LOD values are comparable with those values reported for amperometric methods [15,16], which are usually much more sensitive than other approaches [18,39].

4. Conclusions

In this work, OPH mutant (S5) was successfully displayed on the cell surface of *E. coli*, using a functional truncated INP motif. The whole cell biocatalyst exhibited excellent OPH activity for paraoxon which is the highest among the surface displayed OPH reported previously. Compared with free S5 variant, the cell surface displayed S5 showed good thermostability with an optimal temperature of 55 °C for OPH activity. In addition, the whole cell could retain over 50% of its initial activity within pH 6.5–9. As a whole, the resulting recombinant strain is more stable and robust than purified OPH in terms of long-term stability and facilitation of the protein purification. The prepared OPH-displayed bacteria

were used to develop a novel method for the monitoring of paraoxon, parathion and methyl parathion. This approach showed a wide linear range within 0.5–150 μM for paraoxon, 1–200 μM for parathion and 2.5–200 μM for methyl parathion and a low detection limit of 0.2 μM, 0.4 μM and 1 μM for paraoxon, parathion and methyl parathion, respectively ($S/N=3$). Considering the above-mentioned excellent features, the prepared OPH-displayed bacteria are promising for sensitive detection and fast remediation of pollution caused by specific OPs.

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References

- [1] Mulbry WW, Karns JS. Parathion hydrolase specified by the *Flavobacterium opd* gene: relationship between the gene and protein. *J Bacteriol* 1989;171: 6740–6.
- [2] Serdar CM, Murdock DC, Rohde MF. Parathion hydrolase gene from *Pseudomonas diminuta* MG: subcloning, complete nucleotide sequence, and expression of the mature portion of the enzyme in *Escherichia coli*. *Nat Biotechnol* 1989;7:1151–5.
- [3] Singh BK, Walker A. Microbial degradation of organophosphorus compounds. *FEMS Microbiol Rev* 2006;30:428–71.
- [4] Lei Y, Mulchandani P, Chen W, Mulchandani A. Biosensor for direct determination of fenitrothion and EPN using recombinant *Pseudomonas putida* JS444 with surface-expressed organophosphorous hydrolase. 2. Modified carbon paste electrode. *Appl Biochem Biotech* 2007;136:243–50.
- [5] Lei Y, Mulchandani P, Chen W, Mulchandani A. Biosensor for direct determination of fenitrothion and EPN using recombinant *Pseudomonas putida* JS444 with surface expressed organophosphorus hydrolase. 1. Modified Clark oxygen electrode. *Sensors-Basel* 2006;6:466–72.
- [6] Raushel FM. Bacterial detoxification of organophosphate nerve agents. *Curr Opin Microbiol* 2002;5:288–95.
- [7] Allard AS, Neilson AH. Bioremediation of organic waste sites: a critical review of microbiological aspects. *Int Biodegr Biodegr* 1997;39:253–85.
- [8] Singh BK. Organophosphorus-degrading bacteria: ecology and industrial applications. *Nat Rev Microbiol* 2009;7:156–64.
- [9] Aubert SD, Li Y, Raushel FM. Mechanism for the hydrolysis of organophosphates by the bacterial phosphotriesterase. *Biochemistry* 2004;43:5707–15.
- [10] Sanchez ME, Mendez R, Gomez X, Martin-Villacorta J. Determination of diazinon and fenitrothion in environmental water and soil samples by HPLC. *J Liq Chromatogr R T* 2003;26:483–97.
- [11] Cho Y, Matsuoka N, Kamiya A. Determination of organophosphorus pesticides in biological samples of acute poisoning by HPLC with diode-array detector. *Chem Pharm Bull* 1997;45:737–40.
- [12] Kim DH, Heo GS, Lee DW. Determination of organophosphorus pesticides in wheat flour by supercritical fluid extraction and gas chromatography with nitrogen-phosphorus detection. *J Chromatogr A* 1998;824:63–70.
- [13] Khan IAT, Riazuddin Parveen Z, Ahmed M. Multi-residue determination of synthetic pyrethroids and organophosphorus pesticides in whole wheat flour using gas chromatography. *Bull Environ Contam Toxicol* 2007;79:454–8.
- [14] Lee JH, Park JY, Min K, Cha HJ, Choi SS, Yoo YJ. A novel organophosphorus hydrolase-based biosensor using mesoporous carbon and carbon black for the detection of organophosphate nerve agents. *Biosens Bioelectron* 2010;25:1566–70.
- [15] Deo RP, Wang J, Block I, Mulchandani A, Joshi KA, Trojanowicz M, et al. Determination of organophosphate pesticides at a carbon nanotube/organophosphorus hydrolase electrochemical biosensor. *Anal Chim Acta* 2005;530: 185–9.
- [16] Mulchandani P, Chen W, Mulchandani A, Wang J, Chen L. Amperometric microbial biosensor for direct determination of organophosphate pesticides using recombinant microorganism with surface expressed organophosphorus hydrolase. *Biosens Bioelectron* 2001;16:433–7.
- [17] Mulchandani A, Mulchandani P, Kaneva I, Chen W. Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. 1. Potentiometric microbial electrode. *Anal Chem* 1998;70:4140–5.
- [18] Mulchandani A, Kaneva I, Chen W. Biosensor for direct determination of organophosphate nerve agents using recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. 2. Fiber optic microbial biosensor. *Anal Chem* 1998;70:5042–6.
- [19] Shimazu M, Mulchandani A, Chen W. Cell surface display of organophosphorus hydrolase using ice nucleation protein. *Biotechnol Progr* 2001;17:76–80.
- [20] Shimazu M, Nguyen A, Mulchandani A, Chen W. Cell surface display of organophosphorus hydrolase in *Pseudomonas putida* using an ice-nucleation protein anchor. *Biotechnol Progr* 2003;19:1612–4.
- [21] Richins RD, Kaneva I, Mulchandani A, Chen W. Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. *Nat Biotechnol* 1997;15:984–7.
- [22] Takayama K, Suye S, Kuroda K, Ueda M, Kitaguchi T, Tsuchiyama K, et al. Surface display of organophosphorus hydrolase on *Saccharomyces cerevisiae*. *Biotechnol Progr* 2006;22:939–43.
- [23] Kang DG, Kim JYH, Cha HJ. Enhanced detoxification of organophosphates using recombinant *Escherichia coli* with co-expression of organophosphorus hydrolase and bacterial hemoglobin. *Biotechnol Lett* 2002;24:879–83.
- [24] Chen W, Brühlmann F, Richins RD, Mulchandani A. Engineering of improved microbes and enzymes for bioremediation. *Curr Opin Biotechnol* 1999;10:137–41.
- [25] Yang C, Liu R, Yuan Y, Liu J, Cao X, Qiao C, et al. Construction of a green fluorescent protein (GFP)-marked multifunctional pesticide-degrading bacterium for simultaneous degradation of organophosphates and gamma-hexachlorocyclohexane. *J Agric Food Chem* 2013;61:1328–34.
- [26] Mulchandani A, Rajesh. Microbial biosensors for organophosphate pesticides. *Appl Biochem Biotechnol* 2011;165:687–99.
- [27] Lei Y, Mulchandani P, Wang J, Chen W, Mulchandani A. Highly sensitive and selective amperometric microbial biosensor for direct determination of *p*-nitrophenyl-substituted organophosphate nerve agents. *Environ Sci Technol* 2005;39:8853–7.
- [28] Roodveldt C, Tawfik DS. Directed evolution of phosphotriesterase from *Pseudomonas diminuta* for heterologous expression in *Escherichia coli* results in stabilization of the metal-free state. *Protein Eng Des Sel* 2005;18:51–8.
- [29] Li L, Liang B, Shi JG, Li F, Mascini M, Liu AH. A selective and sensitive D-xylose electrochemical biosensor based on xylose dehydrogenase displayed on the surface of bacteria and multi-walled carbon nanotubes modified electrode. *Biosens Bioelectron* 2012;33:100–5.
- [30] Li L, Liang B, Li F, Shi JG, Mascini M, Lang QL, et al. Co-immobilization of glucose oxidase and xylose dehydrogenase displayed whole cell on multiwalled carbon nanotube nanocomposite films modified-electrode for simultaneous voltammetric detection of D-glucose and D-xylose. *Biosens Bioelectron* 2013;42:156–62.
- [31] Liang B, Li L, Tang XL, Lang QL, Wang HW, Li F, et al. Microbial surface display of glucose dehydrogenase for amperometric glucose biosensor. *Biosens Bioelectron* 2013;45:19–24.
- [32] Liang B, Lang Q, Tang X, Liu A. Simultaneously improving stability and specificity of cell surface displayed glucose dehydrogenase mutants to construct whole-cell biocatalyst for glucose biosensor application. *Bioresour Technol* 2013;147:492–8.
- [33] Xia L, Liang B, Li L, Tang XJ, Palchetti I, Mascini M, et al. Direct energy conversion from xylose using xylose dehydrogenase surface displayed bacteria based enzymatic biofuel cell. *Biosens Bioelectron* 2013;44:160–3.
- [34] Shimazu M, Mulchandani A, Chen W. Simultaneous degradation of organophosphorus pesticides and *p*-nitrophenol by a genetically engineered *Moraxella* sp. with surface-expressed organophosphorus hydrolase. *Biotechnol Bioeng* 2001;76:318–24.
- [35] Lee JS, Shin KS, Pan JG, Kim CJ. Surface-displayed viral antigens on *Salmonella* carrier vaccine. *Nat Biotechnol* 2000;18:645–8.
- [36] Zhang H, Li Q, Ye T, Zhang Z, Li L. Optimization of the whole-cell catalytic activity of recombinant *Escherichia coli* cells with surface-immobilized organophosphorus hydrolase. *J Environ Biol* 2013;34:315–9.
- [37] Cheng YD, Karns JS, Torrents A. Characterization of a phosphotriesterase from genetically-engineered *Escherichia coli*. *J Environ Sci Health B* 1998;33:347–67.
- [38] Yang C, Freudl R, Qiao C, Mulchandani A. Cotranslocation of methyl parathion hydrolase to the periplasm and of organophosphorus hydrolase to the cell surface of *Escherichia coli* by the Tat pathway and ice nucleation protein display system. *Appl Environ Microbiol* 2010;76:434–40.
- [39] Mulchandani A, Mulchandani P, Chauhan S, Kaneva I, Chen W. A potentiometric microbial biosensor for direct determination of organophosphate nerve agents. *Electroanalysis* 1998;10:733–7.