Surface Display of Organophosphorus Hydrolase on *Saccharomyces cerevisiae*

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The gene encoding organophosphorus hydrolase (OPH) from *Flavobacterium* species was expressed on the cell surface of *Saccharomyces cerevisiae* MT8-1 using a glycosylphosphatidylinositol (GPI) anchor linked to the C-terminal region of OPH. Immunofluorescence microscopy confirmed the localization of OPH on the cell surface, and fluorescence intensity measurement of cells revealed that 1.4×10^4 molecules of OPH per cell were displayed. Seventy percent of OPH whole-cell activity was detected on the cell surface by protease accessibility assay. The activity of OPH was highly dependent on cell growth conditions. The maximum activity was obtained when cells were grown in a synthetic dextrose medium lacking tryptophan (SD-W) buffered by 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, 200 mM, pH 7.0) at 20 °C, and cobalt chloride was added at 0.1 mM. *S. cerevisiae* MT8-1 displaying OPH which exhibited a higher activity than *Escherichia coli* displaying OPH using the ice nucleation protein (INP) anchor. The use of *S. cerevisiae* MT8-1, which has a "generally regarded as safe (GRAS)" status, as a host for the easy expression of the OPH gene provides a new biocatalyst useful for simultaneous detoxification and detection of organophosphorus pesticides.

Introduction

Organophosphorus compounds (OPs) are widely used as pesticides, fungicides, herbicides, and chemical warfare agents. Repeated or prolonged exposure to these extremely toxic substances can cause delayed cholinergic toxicity and neurotoxicity (*1*−*3*). In Japan, highly toxic OPs, such as parathion, have been detected in imported agricultural food products. The growing public concern about their safety and the widespread use of OPs in modern agriculture has stimulated the development of methodologies for the detoxification of OPs. Current techniques for detoxifying organophosphate pesticides include harsh chemical treatment, incineration, and landfills. These processes also lead to serious environmental pollution.

Organophosphorus hydrolase (OPH) effectively hydrolyzes a range of organophosphate esters, including pesticides, such as parathion, coumaphos, and acephate, and chemical warfare agents, such as soman, sarin, VX, and tabun (*1*, *4*−*9*). The catalytic hydrolysis of each molecule of these compounds releases two protons. The measurement and correlation of released protons to OP concentration form the basis of the use of a potentiometric enzyme electrode, and OPH-modified potentiometric enzyme electrodes have been developed (*10*). The use of whole cells has been explored as an alternative biological catalyst without the high cost of purifying enzymes. Although microbial biosensors for OPs have been developed using recombinant *Escherichia coli* cells expressing the OPH gene intracellularly, the response was slow due to the mass-transport limitation of substrates and products across the cell membrane (*11*). The same problem also occurs for the OPH detoxification reactor system using these recombinant cells. To overcome this drawback, *E. coli* cells that express the OPH gene on the surface were genetically engineered. Both the Lpp-OmpA (*12*, *13*) and INPNC anchors (*14*) were applied to express the OPH gene on the surface of *E. coli* with significantly improved efficiencies.

Saccharomyces cerevisiae, the common bakers' yeast, is another potential microorganism that can be engineered to target onto the cell surface. Yeast is particularly attractive because it is generally considered to be safe and, as a eukaryote, it offers a protein quality control system via the secretory pathway in the folding of displaying proteins. In this paper, we describe the construction of live biocatalysts for organophosphorus pesticide detoxification and detection based on recombinant *Saccharomyces cerevisiae* cells expressing the OPH gene using the glycosylphosphatidylinositol (GPI) anchor system, in which the secretion signal sequence of α-agglutinin and a GPI anchor attachment signal sequence have been genetically fused, respectively, to the N- and C-terminal regions of the target protein (*15*).

Materials and Methods

Strains, Media, and Culture Conditions. *Escherichia coli* strain DH5α [F-, *endA1*, *hsdR17* ($r_K-m_K^+$), *supE44*, *thi-1*, λ^-, *rec A1*, *gyrA96*, Δ*lacU169* (Φ*80lacZ*Δ*M15*)] (*16*) was used as a host for recombinant DNA manipulation. *Saccharomyces cerevisiae* strain MT8-1 (*MATa*, *ade*, *his3*, *leu2*, *trp1*, *ura3*)

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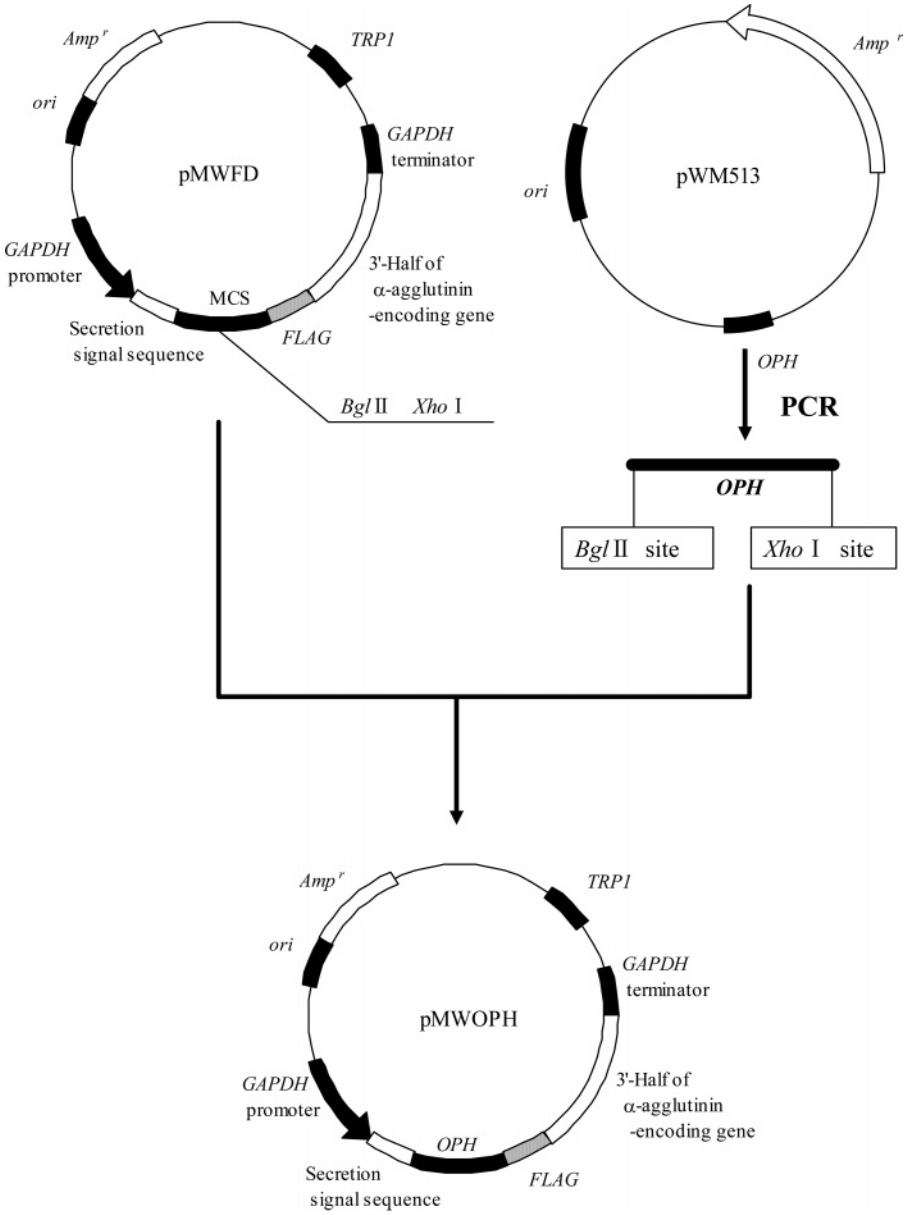


Figure 1. Plasmid pMWOPH constructed for display of organophosphate hydrolase protein (OPH) on yeast cell surface. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase. The FLAG-encoding gene was fused for the detection of successful display of OPH on the yeast cell surface.

was used to display OPH (*17*). The Luria−Bertani (LB) medium (1% w/v tryptone, 0.5% w/v yeast extract, 0.5% w/v sodium chloride, 0.1% w/v glucose, supplemented with 50 μg/mL of ampicillin) was used for bacterial growth and plasmid amplification. Yeast recombinant transformants were selected on the SD-W medium (0.67% w/v yeast nitrogen base without amino acids, 2% w/v glucose, 0.002% w/v adenine sulfate, 0.002% w/v L-histidine-HCl, 0.003% w/v L-leucine, and 0.002% w/v uracil) plates. HEPES was added to the liquid SD-W medium at a concentration of 200 mM (pH 7.0). Cells were grown in 500 mL flasks in a Bio-Shaker BR300LF (Taitec Co., Japan) with vigorous agitation (300 rpm) at 20, 25, or 30 °C. Cobalt chloride was added to the medium during the initial exponential growth phase. Cell growth in the culture broth was measured on the basis of absorbance at 600 nm. Cells were harvested at appropriate times after the stationary phase.

Construction of Plasmids and Transformation of Yeast. The plasmid pMWOPH for displaying OPH under the control of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter was constructed as shown in Figure 1. A DNA fragment containing the region encoding the OPH gene from *Flavobacterium* species was prepared by PCR using the two primers 5′-TAGAAGATCTTCGATCGGCACAGGCGATCGGATCAAT-3′ and 5′-CACCCTCGAGTGACGCCCGCAAGGTCGGTGACAA-3′ (underlined: *Bgl* II and *Xho* I sites, respectively) with the pWM513 plasmid as the template (*18*) and was then digested by *Bgl* II and *Xho* I. The PCR product was inserted into the same sites of the multicloning site in the low-copy-number yeast cell-surface-displaying cassette vector pMWFD. The pMWFD contains the gene encoding the secretion signal of glucoamylase under the control of the GAPDH promoter. The resulting plasmid was named pMWOPH. This plasmid allows the surface display of OPH on *S. cerevisiae* MT8-1 cells through the GPI anchor linked at the C terminus of OPH. A FLAG^R tag was also included between the C terminus of OPH and the anchor to allow easy probing with anti-FLAG antisera. The plasmid was transformed into *S. cerevisiae* MT8-1 by the lithium acetate method. The negative control strain was transformed with pICAS (*19*), which is the chromosome-integrated-type vector consisting of the GAPDH promoter, secretion-signal-sequence-encoding gene, and anchoring-protein-encoding gene for cell-surface display.

Measurement of OPH Whole-Cell Activity. Yeast cells harboring pMWOPH or pICAS were grown under appropriate conditions to an OD_{600} between 0.5 and 1.0. Yeast cells were centrifuged at 18 000*g* for 1 min, and collected cells were washed three times with HEPES (200 mM, pH 7.0) buffer and suspended in HEPES (50 mM, pH 8.5) buffer with 50 μM cobalt chloride. Activity assays were conducted in 1.5 mL disposable tubes. For each assay, 50 μL of 20 mM paraoxon (Sigma, St. Louis, MO) dissolved in 10% methanol was added to a 950 μL cell suspension (OD_{600} = 1.0) to obtain a final concentration of 1 mM. Reaction mixtures were incubated at 30 °C. The amount of *p*-nitrophenol produced by hydrolysis was measured using a HPLC 7000 system (Hitachi Co., Tokyo, Japan) connected to a ODS 80Ts column (Tosoh Co., Tokyo, Japan). At 0 and 24 h after the reaction, aliquots of 200 μL were taken from the reaction mixture and filtered with a Millipore membrane (0.25 μm pore size). An aliquot (20 μL) of an obtained sample was subjected to HPLC to quantify *p*-nitrophenol content. The absorbance of *p*-nitrophenol was measured at 410 nm (E_{410} = 16 500 M^{-1} cm^{-1}). OPH activity was expressed as nanomoles of paraoxon hydrolyzed per minute (*U*), per OD_{600nm} whole cells.

Immunostaining, Fluorescence Microscopy, Fluorometric Assay, Flow Cytometry, and Proteinase Accessibility Assay. The immunofluorescent labeling of yeast cells was carried out as follows. A cell suspension (OD_{600} = 1.0) was centrifuged at 18 000*g* for 1 min, and collected cells were washed with potassium phosphate buffer ((KPB), 50 mM, pH 7.2) and suspended in 3.7% formaldehyde KPB (50 mM, pH 7.2) for 1.5 h. The cells were washed three times with KPB (50 mM, pH 7.2). The cells were blocked with 1% BSA in 300 μL of PBS (10 mM Na_2HPO_4, 1.76 mM NaH_2PO_4, 137 mM NaCl, and 2.7 mM KCl, pH 7.4) for 30 min at room temperature. Anti-FLAG IgG (1 μL) (Sigma, St. Louis, MO) was added to the cell suspension and incubated at room temperature for 1.5 h. The cells were then washed with PBS, centrifuged, suspended in 300 μL of PBS with a secondary antibody (1:300 dilution), Alexa Fluor TM 488-conjugated goat anti-mouse IgG (Molecular Probes, Eugene, OR), and incubated at room temperature for 1.5 h. After washing with PBS, the cells were observed under a Leica TCS-SP2 AOBS confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany) equipped with a UV 63 × 1.4−0.60 NA oil immersion PlanApo objective. The excitation and emission wavelengths used were 488 and 510−535 nm, respectively.

A universal plate reader (Perkin-Elmer Life and Analytical Sciences, Boston, MA) was used to measure the fluorescence intensity of samples in PBS on 96-well plates. A filter with an excitation wavelength of 485 nm and another filter with an emission wavelength of 535 nm were employed to detect fluorescence at room temperature. After the fluorescence intensity of cell-surface-displayed OPH was measured, the number of OPH molecules on the cell surface was determined according to a calibration curve prepared with the anti-FLAG antibody.

The number of yeast cells displaying OPH was determined using an EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA) with a 488 nm excitation wavelength and a 525 nm emission wavelength to estimate the percentage of OPH molecules displayed.

Yeast cells harboring pMWOPH were suspended in a 1 mL suspension containing 15% w/v sucrose, 15 mM Tris-HCl, 0.1 mM EDTA, pH 7.8. Samples were incubated for 1 h with 5 μL of 20 mg/mL proteinase-K (Nakarai Tesque, Kyoto, Japan) at room temperature. To inhibit further proteinase-K activity, 10 μM phenylmethylsulfonylfluoride (Nakarai Tesque, Kyoto, Japan) was added. Proteinase-K-treated and -untreated yeast cells were assayed for OPH whole-cell activity as described above.

Table 1. Effect of Temperature and Cobalt Addition on OPH Activity of *S. cerevisiae* MT8-1 Carrying pMWOPH

	Temperature (°C)[b]			Co^{2+} Concentration (mM)[c]		
	20	25	30	0	0.1	1.0
activity[a]	130	69	63	26	130	100

[a] Nanomoles of parathion hydrolyzed per minute (*U*) per OD_{600} whole cells. [b] Co^{2+} concentration was 0.1 mM. [c] Temperature was set at 20 °C.

Plasmid Stability Studies. Yeast cells carrying pMWOPH were grown in the SD-W medium buffered with HEPES (200 mM, pH 7.0). A sample was collected at 48 h. After appropriate dilutions were made, 100 μL of the sample was plated on the SD and SD-W media. Yeast colonies were counted after 1 or 2 days of incubation at 30 °C. Plasmid stability was determined as the ratio of the number of colonies formed on SD and SD-W plates.

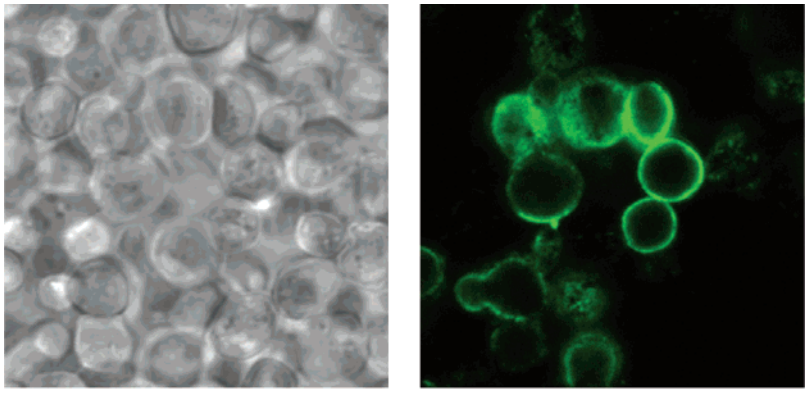
Results and Discussion

Effects of Culture Conditions on OPH Whole-Cell Activity. The effect of pH was investigated by culturing yeast cells harboring pMWOPH in the SD-W medium and SD-W medium buffered with HEPES at pH 7.0. OPH whole-cell activity was observed only in the SD-W-buffered medium (data not shown). To examine the effect of cultivation temperature on cell surface expression level, the recombinant cells were grown at either 20, 25, or 30 °C for 2 days until the stationary phase; the highest OPH activity was obtained at 20 °C (Table 1). We considered that constitutive expression at or higher than 25 °C may impair the protein folding mechanism. On the other hand, a lower temperature might retard the premature folding of the fusion protein, favoring the proper translocation and high activities of OPH.

Addition of a low concentration of cobalt chloride, which favors the formation of an active metal center, was also effective for improving OPH activity (*20*). OPH whole-cell activity was enhanced by approximately 5-fold following the addition of 0.1 mM cobalt chloride at the initial exponential phase (Table 1). The optimal OPH whole-cell activity (130 U/OD_{600}) was 5-fold higher than those reported for *E. coli* displaying OPH on the cell surface (Table 2) (*14*).

Detection of OPH on Cell Surface. To confirm the localization of OPH on the cell surface, yeast cells were immunolabeled with the anti-FLAG antibody and Alexa Fluor 546-conjugated goat anti-mouse IgG. Cells harboring pMWOPH were brightly fluorescent, indicating that OPH was successfully displayed on the cell surface using the α-agglutinin-GPI anchor (Figure 2). Fluorescence was not detected from the surface of pICAS-transformed yeast cells as a control. Because these antibodies cannot access the interior of a yeast cell under the labeling conditions employed here, our results confirmed that OPH was indeed successfully displayed on the yeast cell surface.

Protease accessibility experiments were also carried out to ascertain the presence of enzymatically active OPH on the cell surface. As proteinase-K cannot readily diffuse across the cell membrane, degradation should only occur with proteins completely exposed (not buried) on the surface. A 70% reduction in OPH whole-cell activity was observed in cells harboring pMWOPH incubated with proteinase-K. It appears that a substantial fraction of active OPH is completely exported across



A B

Figure 2. Immunofluorescence labeling of transformed cells using anti-FLAG antibody and Alexa 488-conjugated goat anti-mouse IgG. Phase contrast micrograph (A) and immunofluorescence (B) of transformed *S. cerevisiae* MT8-1 with pMWOPH for display of OPH.

Table 2. Evaluation of Yeast and *E. coli* Strains with OPH on Surface Using Various OPH-Displaying Systems

strain (plasmid)	anchor system	activity[a]
yeast MT8-1 (pMWOPH)	GPI	130
E. coli XL1-blue (pINCOP)	InaV	18[b]
E. coli XL1-blue (pIKNCOP)	InaK	15[b]
E. coli XL1-blue (pOP131)	Lpp-OmpA	3[b]

[a] Nanomoles of parathion hydrolyzed per minute (*U*) per OD_{600} whole cells. [b] Data are cited from ref 14.

the membrane and anchored onto the cell surface, although OPHs buried in the cell wall were not evaluated.

Quantification of OPH on Cell Surface. The number of OPH molecules displayed on the cell surface was calculated by measuring the fluorescence intensity of a cell pellet suspended in PBS (pH 7.0) using a fluorometer. A standard curve was prepared using known amounts of Alexa Fluor 546-conjugated goat anti-mouse IgG. The following formula was obtained from the standard curve and used for the calculation:

$$\text{number of OPH molecules displayed per cell} = \{RFU_f \times 0.945 \times 10^7\}/1 \times 10^7$$

where RFU_f (relative fluorescence unit) is an arbitrary unit measured with a fluorometer under the conditions described in Materials and Methods and 1 $OD_{600} = 1 \times 10^7$ cells/mL. The number of OPH molecules displayed on the cell surface of *S. cerevisiae* strain MT8-1 harboring pMWOPH was estimated to be 1.4×10^4, where the yeast cells were cultivated in optimum conditions. The number of cell-surface-expressed OPH molecules was also calculated on the basis of the specific activity of purified OPH at 8020 units/mg (one unit of activity is defined as the hydrolysis of 1.0 μmol of paraoxon/min) (*20*). The estimated number of 4.6×10^3 obtained is consistent with the result obtained from the fluorescence intensity measurement. If we assume that yeast cells are spheres and OPH molecules have a 5×10^{-3} μm radius (*21*), approximately 10^6 OPH molecules can be closely packed on the yeast cell surface. Therefore, it may still be possible to further increase by 10^5 the number of OPH molecules anchored per cell.

However, flow cytometry showed that only 10% of the total population expressed OPH on the cell surface. One reason for the low percentage may be the incomplete translocation of OPH to the surface across the cell wall, that is, the major proportion of OPH may be buried in the cell wall. The insertion of a linker peptide (spacer) consisting of the Gly/Ser repeat sequence at the C-terminal portion of *Rhizopus orizae* lipase has been reported to enhance lipase activity on the cell surface (*22*). Thus, the insertion of an appropriate length of spacer may be effective in the full display of OPH. In addition, the use of KDH-3(ΔKex2) species as a host cell may also improve OPH display because a sequence similar to the Kex2 endopeptidase-cleaving site was found to be present in the open reading frame sequence of OPH.

Plasmid stability is also a potential reason for the low percentage of active cells. A substantial decline in the number of plasmid-bearing cells with only 50% of the cells containing the plasmid after 48 h cultivation in the SD-W medium was observed. Therefore, an alternative expression system utilizing a chromosome-integrated multicopy-number vector may be effective for solving this problem.

Stability of Cultures Displaying OPH. To test whether the constitutive expression of OPH/α-agglutinin-GPI inhibits cell growth, the growth kinetics of cells carrying pMWOPH and that of cells carrying pICAS were compared. The obvious growth inhibition was not observed as both cultures showed the same final cell density after 48 h of incubation (Figure 3).

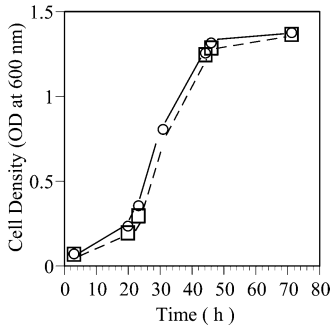


Figure 3. Cell growth kinetics of *S. cerevisiae* MT8-1 harboring pICAS (□) and pMWOPH (○). Optical density was monitored at 660 nm.

Conclusion

In this study, we have successfully demonstrated the functional display of OPH on the yeast cell surface. Yeast cells exhibited a higher potential for the OPH-displaying system than *E. coli*. Surface-engineered yeast cells are safe to use and are repeatedly usable as a biocatalyst. The current technology is very useful not only for the detoxification OP nerve agents but also for the rapid detection of OPs. For example, it is well-known that the fluorescence intensity of the green fluorescent protein (GFP) is highly dependent on pH; a 65% attenuation in fluorescence of GFP for a pH change from 8 to 6 has been

reported for GFP expressed on the surface of *E. coli* (*23*). Thus, it is possible to detect the presence of OP compounds by monitoring the changes in fluorescence of GFP based on the protons generated by the hydrolysis by OPH by coexpressing GFP with OPH on yeast surface. This work is currently in progress in our laboratory.

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References and Notes

(1) Donarski, W. J.; Dumas, D. P.; Heitmeyer, D. P.; Lewis, V. E.; Raushel, F. M. Structure−activity relationships in the hydrolysis of substrates by the phosphotriesterase from *Pseudomonas diminuta*. *Biochemistry* **1989**, *28*, 4650−4655.

(2) Chapalamadugu, S.; Chaudhry, G. S. Microbiological and biotechnological aspects of metabolism of carbamates and organophosphates. *Crit. Rev. Biotechnol*. **1992**, *12*, 357−389.

(3) Tuovinen, K.; Kalistekorhonen, E.; Raushel, F. M.; Hanninen, O. Phosphotriesterase promising candidate for use in detoxification of organophosphates. *Fundam. Appl. Toxicol.* **1994**, *23*, 578−584.

(4) Dumas, D. P.; Wild, J. R.; Raushel, F. M. Diisopropylfluorophosphate hydrolysis by a phosphotriesterase from *Pseudomonas diminuta*. *Biotechnol. Appl. Biochem.* **1989**, *11*, 235−243.

(5) Dumas, D. P.; Durst, H. D.; Landis, W. G.; Raushel, F. M.; Wild, J. R. Inactivation of organophosphorus nerve agents by the phosphotriesterase from *Pseudomonas diminuta*. *Arch. Biochem. Biophys*. **1990**, *277*, 155−159.

(6) Dumas, D. P.; Caldwell, S. P.; Wild, J. R.; Raushel, F. M. Purification and properties of the phosphotriesterase from *Pseudomonas diminuta*. *J. Biol. Chem.* **1989**, *33*, 19659−19665.

(7) Lewis, V. E.; Donarski, W. J.; Wild, J. R.; Raushel, F. M. Mechanism and stereochemical course at phosphorus of the reaction catalyzed by a bacterial phosphotriesterase. *Biochemistry* **1988**, *27*, 1591−1597.

(8) Dave, K. I.; Miller, C. E.; Wild, J. R. Characterization of organophosphorus hydrolases and the genetic manipulation of the phosphotriesterase from *Pseudomonas diminuta*. *Chem. Biol. Interact.* **1993**, *87*, 55−68.

(9) Lai, K.; Dave, K. I.; Wild, J. R. Bimetallic binding motifs in organophosphorus hydrolase are important for catalysis and structural organization. *J. Biol. Chem.* **1994**, *269*, 16579−16584.

(10) Mulchandani, P.; Mulchandani, A.; Kaneva, I.; Chen, W. Biosensor for direct determination of organophosphate nerve agents, 1. Potentiometric enzyme electrode. *Biosens. Bioelectron*. **1999**, *14*, 75−85.

(11) Rainina, E. I.; Efremenco, E. N.; Varfolomeyev, S. D.; Simonian, A. L.; Wild, J. R. The development of a new biosensor based on recombinant *E. coli* for the direct detection of organophosphorus neurotoxins. *Biosens. Bioelectron.* **1996**, *11*, 991−1000.

(12) Richins, R. D.; Kaneva, I.; Mulchandani, A.; Chen, W. Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. *Nat. Biotechnol.* **1997**, *15*, 984−987.

(13) Kaneva, I.; Mulchandani, A.; Chen, W. Factors influencing parathion degradation by recombinant *Escherichia coli* with surface-expressed organophosphorus hydrolase. *Biotechnol. Prog*. **1998**, *14*, 275−278.

(14) Shimazu, M.; Mulchandani, A.; Chen, W. Cell surface display of organophosphorus hydrolase using ice nucleation protein. *Biotechnol. Prog*. **2001**, *17*, 76−80.

(15) Ueda, M.; Tanaka, A. Cell surface engineering of yeast: Construction of arming yeast with biocatalyst. *J. Biosci. Bioeng.* **2000**, *90*, 125−136.

(16) Hanahan D. Studies on transformation of *Escherichia coli* with plasmid. *J. Mol. Biol.* **1983**, *166*, 557−580.

(17) Tajima, M.; Nogi, Y.; Fukasawa, T. Primary structure of the *Saccharomyces cerevisiae* GAL7 gene. *Yeast* **1985**, *1*, 67−77.

(18) Mulbry, W. W.; Karns, J. S. Parathion hydrolase specified by the *Flavobacterium* opd gene: Relationship between the gene and protein. *J. Biotechnol*. **1989**, *171*, 6740−6746.

(19) Ye, K.; Shibasaki, S.; Ueda, M.; Murai, T.; Kamasawa, N.; Osumi, M.; Shimizu, K.; Tanaka, A. Construction of an engineered yeast with glucose-inducible emission of green fluorescence from the cell surface. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 90−96.

(20) Omburo, G. A.; Kuo, J. M.; Mullins, L. S.; Raushel, F. M. Characterization of the zinc binding site of bacterial phosphotriesterase. *J. Biol. Chem.* **1992**, *267*, 13278−13283.

(21) Benning, M. M.; Kuo, J. M.; Raushel, F. M.; Holden, H. M. Three-dimensional structure of phosphotriesterase: An enzyme capable of detoxifying organophosphate nerve agents. *Biochemistry* **1994**, *33*, 15001−15007.

(22) Washida, M.; Takahashi, S.; Ueda, M.; Tanaka, A. Spacer-mediated display of active lipase on the yeast cell surface. *Appl. Microbiol. Biotechnol*. **2001**, *56*, 681−686.

(23) Shi, H.; Su, W. W. Display of green fluorescent protein on *Escherichia coli* cell surface. *Enzyme Microb. Technol*. **2001**, *28*, 25−34.

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