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# Mussel adhesive protein-based whole cell array biosensor for detection of organophosphorus compounds

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ABSTRACT

A whole cell array biosensor for the efficient detection of neurotoxic organophosphate compounds (OPs) was developed through the immobilization of recombinant *Escherichia coli* cells containing periplasmic-expressing organophosphorus hydrolase (OPH) onto the surface of a 96-well microplate using mussel adhesive protein (MAP) as a microbial cell-immobilizing linker. Both the paraoxon-hydrolyzing activity and fluorescence microscopy analyses demonstrated that the use of MAP in a whole cell biosensor increased the cell-immobilizing efficiency and enhanced the stability of immobilized cells compared to a simple physical adsorption-based whole cell system. Scanning electron microscopic analyses also showed that the *E. coli* cells were effectively immobilized on the MAP-coated surface without any pretreatment steps. The whole cell array biosensor system, prepared using optimal MAP coating (50 $\mu g/cm^2$) and cell loading (4 $OD_{600}$), detected paraoxon levels as low as 5 μM with high reproducibility, and its quantitative detection range was ~5–320 μM. The MAP-based whole cell array biosensor showed a good long-term stability for 28 day with 80% retained activity and a reusability of up to 20 times. In addition, paraoxon in tap water was also successfully detected without a reduction in sensitivity. Our results indicate that the proposed MAP-based whole cell array system could be used as a potential platform for a stable and reusable whole cell biosensor.



## 1. Introduction

Enzymes are often used as biocatalysts in biosensors due to their high specific activity (Inaba et al., 2003; Jamal et al., 2010; Park et al., 2011; Savizi et al., 2012). Whole cell biocatalysts offer several advantages over free enzymes, including high stability, reduced purification requirements, low preparation cost, and efficient cofactor regeneration (de Carvalho, 2011). Whole cell-based biosensors are used for high-throughput drug discovery, clinical diagnosis, and environmental monitoring of chemicals and heavy metals (Kumar and D'Souza, 2011a; Liu et al., 2007, 2011; Mulchandani et al., 2001; Perdikaris et al., 2011). As a key factor in the development of a whole cell biosensor, the surface immobilization technique can affect both sensitivity and stability (Alvarez et al., 2009; Braschler et al., 2005; Jen et al., 1996; Jha et al., 2009; Kumar and D'Souza, 2010, 2011a, 2011b). Physical (entrapment or adsorption) and chemical (crosslinking) methods have been widely used for surface immobilization of cells (D'Souza, 2001). Physical immobilization methods, in particular, have several limitations. The mass transfer problems occur when cells are entrapped using polymer gels, such as polyacrylamide, alginate, and agarose (Braschler et al., 2005; Jen et al., 1996), and cell immobilization using direct adsorption or adhesion may lead to the loss of cells from surface during continuous use (D'Souza, 2001). While chemical crosslinking methods can overcome the limitations of physical adsorption/adhesion (Kumar and D'Souza, 2010, 2011a, 2011b), cell viability may be negatively affected (D'Souza, 2001; Lei et al., 2006). Thus, improved immobilization techniques with both biocompatibility and strong surface adhesion would assist in the development of whole cell biosensors.

Mussel adhesive proteins (MAPs) from marine mussels are water-insoluble and biocompatible bioadhesives that can adhere tightly to substrata (Cha et al., 2008; Dove and Sheridan, 1986; Waite and Tanzer, 1981). Because MAPs are able to form strong bonds with both biomolecules and diverse surfaces including glass, metal, and plastics without any pretreatment processes (Burzio et al., 1989; Waite, 1987), they have been suggested as immobilizing agents for enzymes, proteins, and antibodies (Burzio et al., 1996; Kim et al., 2011; Saby and Luong, 1998). Our previous work on recombinant MAP in *Escherichia coli* demonstrated that recombinant MAP has both efficient adhesion ability and biocompatibility for various cell types, including mammalian and insect cells (Choi et al., 2010; Hwang et al., 2007a, 2007b, 2007c).

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Organophosphate compounds (OPs) are widely employed as pesticides and insecticides for both agriculture and household use due to their broad-spectrum activities and low cost. However, the overuse of OPs has caused environmental pollution, increasing ecological and human health risks. Some OPs have been also used in chemical warfare as neurotoxins (Donarski et al., 1989). Due to the environmental risk associated with OPs, sensitive, reliable, and cost-effective analytical tools are required to detect toxic OPs. Organophosphorus hydrolase (OPH) is a metalloenzyme produced by the soil microorganisms *Pseudomonas diminuta* and *Flavobacterium* sp. and degrades a broad spectrum of OPs (Grimsley et al., 1997; Lai et al., 1995; McDaniel et al., 1988; Mulbry and Karns, 1989). OPH has been used for the detection of OPs in the environment (Chough et al., 2002; Hossain et al., 2011a, 2011b; Mulchandani et al., 1999; Rogers et al., 1999). Several biosensors based on OPH-expressing cells have also been developed for OP detection (Kumar et al., 2006, Kumar and D'Souza, 2010, 2011a, 2011b; Mulchandani et al., 1998a, 1998b, 1998c, 2001; Rainina et al., 1996).

In this present work, we developed a whole cell array biosensor for the detection of neurotoxic OPs using MAP as a cell-immobilizing linker. We employed the previously engineered *E. coli* as a target whole cell, which expresses OPH in its periplasmic space under the assistance of chaperone to reduce substrate/product diffusion limitation and to increase OP degradation efficiency (Kang et al., 2012). Diverse aspects of the whole cell array biosensor system were evaluated, including quantitative detection limits, reproducibility, long-term stability, and reusability.

## 2. Experimental

### 2.1. Cell culture condition

The recombinant *E. coli* cells expressing OPH in the periplasmic space and GroEL/ES in the cytoplasm (Kang et al., 2012) were cultured in Luria-Bertani (LB) medium (0.5% (w/v) yeast extract, 1% (w/v) tryptophan, and 1% (w/v) NaCl) containing 50 μg/mL of ampicillin (Sigma-Aldrich, St. Louis, MO, USA) and 25 μg/mL of chloramphenicol (Sigma-Aldrich). When the culture reached a cell density ($OD_{600}$) of 1.2, 1 mM (final concentration) isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma-Aldrich), 10 ng/mL tetracycline, and 0.5 mM $CoCl_2$ were added to induce the expression of recombinant OPH and GroEL/ES. The recombinant cells were grown at 37 °C for an additional 12 h. DsRed-expressing recombinant *E. coli* culture was grown to 0.8–0.9 $OD_{600}$ at 37 °C in shake flask containing 50 mL LB medium with 50 μg/mL ampicillin. Protein expression was induced by adding IPTG to a final concentration of 1 mM. The recombinant cells were grown at 37 °C for an additional 10 h. The cells were harvested by centrifugation at 4000 *g* for 10 min

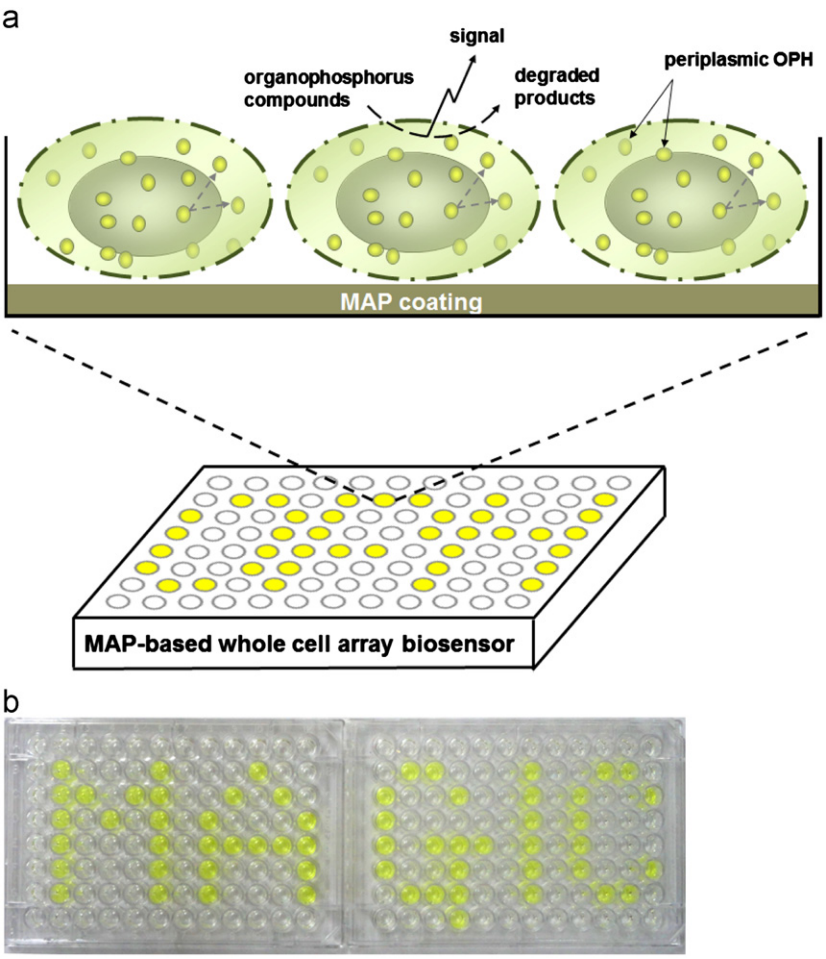


**Fig. 1.** (a) Schematic representation of the whole cell array biosensor for the detection of OPs and (b) demonstration of paraoxon detection as the word 'MAGIC'. Surfaces of the 96-well microplate were coated with MAP and then, recombinant *E. coli* cells expressing OPH in the periplasmic space were immobilized onto the MAP-coated well surfaces.