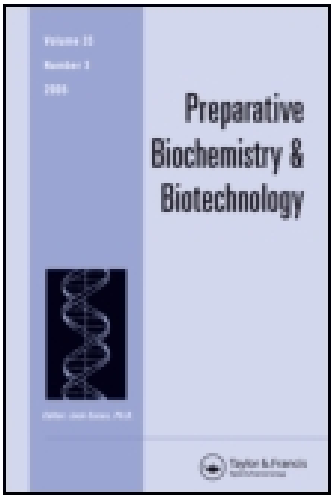

Preparative Biochemistry and Biotechnology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lpbb20

A RECOMBINANT Escherichia coli BIOSENSOR FOR DETECTING POLYCYCLIC AROMATIC HYDROCARBONS IN GAS AND AQUEOUS PHASES

Jae Ho Cho [a] , Da Young Lee [a] , Woon Ki Lim [a] & Hae Ja Shin [b]
[a] Department of Molecular Biology , College of Natural Sciences, Pusan National University , Busan , Republic of Korea
[b] Division of Energy and Bioengineering , Dongseo University , Busan , Republic of Korea

Accepted author version posted online: 12 Mar 2014.Published online: 11 Jul 2014.

To cite this article: Jae Ho Cho , Da Young Lee , Woon Ki Lim & Hae Ja Shin (2014) A RECOMBINANT Escherichia coli BIOSENSOR FOR DETECTING POLYCYCLIC AROMATIC HYDROCARBONS IN GAS AND AQUEOUS PHASES, Preparative Biochemistry and Biotechnology, 44:8, 849-860, DOI: 10.1080/10826068.2014.887577

To link to this article: http://dx.doi.org/10.1080/10826068.2014.887577

Preparative Biochemistry & Biotechnology, 44:849–860, 2014

ISSN: 1082-6068 print/1532-2297 online
DOI: 10.1080/10826068.2014.887577

Taylor & Francis
Taylor & Francis Group

A RECOMBINANT *Escherichia coli* BIOSENSOR FOR DETECTING POLYCYCLIC AROMATIC HYDROCARBONS IN GAS AND AQUEOUS PHASES

Jae Ho Cho,[1] Da Young Lee,[1] Woon Ki Lim,[1] and Hae Ja Shin[2]

[1]*Department of Molecular Biology, College of Natural Sciences, Pusan National University, Busan, Republic of Korea*
[2]*Division of Energy and Bioengineering, Dongseo University, Busan, Republic of Korea*

□ *Recombinant microbial biosensors are known to be simple, cheap, and very efficient monitoring tools for detecting various environmental pollutants in the field. However, although various recombinant microbial biosensors have been developed for aqueous-phase samples, very few are applicable to the gas phase. Here, we report a recombinant* Escherichia coli *biosensor that can be used to monitor polycyclic aromatic hydrocarbons (PAHs) in both gas and aqueous phases by color development. Among the PAHs, naphthalene and salicylate are often used as model compounds, since they are less toxic than other options and they are widely used in various applications. Here, recombinant* E. coli *cells carrying* nahR *(encoding the NahR regulatory protein for naphthalene degradation)::*lac Z *fusion genes were constructed and suspended (for aqueous measurements) or co-immobilized (for gaseous measurements) with chlorophenol red-β-D-galactopyranoside (CPRG). Biosensing was then performed by β-galactosidase, which hydrolyzed CPRG as a substrate, developing detectable red color with the naked eye. The system showed selective responses to salicylate and naphthalene. Importantly, its response to naphthalene was much more sensitive (about 10^5-fold) in the gas phase compared to the aqueous phase. Thus, this system could potentially be used for the instrument-free, color-change-based monitoring of gaseous pollutants.*

Keywords gas-phase pollutants, naphthalene, recombinant *E. coli* biosensor, salicylate

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), which are among the most widespread organic pollutants, are often formed as by-products of petroleum processing and the combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacco, and incense.[1] This pollution is of

Address correspondence to Hae Ja Shin, Division of Energy and Bioengineering, Dongseo University, Busan, 617-716, Republic of Korea. E-mail: hjshin@gdsu.dongseo.ac.kr

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lpbb.

concern because some PAHs have been identified as carcinogens, mutagens, and teratogens.[2]

Current techniques for measuring PAHs (e.g., chromatography) offer precise and accurate detections, but tend to be complicated, expensive, and impractical for field monitoring.[3] In addition, the volatile characteristics of PAHs mean that they are often lost during transport and sample pretreatment, complicating laboratory measurements.[4] PAHs are lipophilic (mix easily with oil) and volatile, show significant gas-phase partitioning, and are relatively insoluble in water. Therefore, simple, cheap, and reliable biosensors, especially those capable of detecting PAHs in both gas and aqueous phases, could be useful alternatives for monitoring PAHs in the field.[4,5]

Numerous recombinant microbial biosensors have been reported for the on-site detection of various environmental pollutants.[6–9] A biosensor typically consists of a biological sensing element that is capable of recognizing a target molecule (analyte) in the environment, and a transducer that transmits a signal when the sensing element binds its specific target molecule.[10,11] Such signals could be optical, electrochemical, acoustic, or electronic.[12,13] The sensitivities and specificities of the existing microbial biosensors have been increased by genetic engineering,[14–18] but most of them still require bulky transducers, such as for spectrophotometers, luminometers, fluorometers, amperometers, and so on. In addition, almost all of these techniques detect target molecules in the aqueous phase, not in the gas phase.

With respect to gas-phase measurements, a filter-immobilized bioluminescent *Pseudomonas putida* strain carrying the NAH7 plasmid and a chromosomally inserted gene fusion between the *sal* promoter and the *luxAB* genes was previously used to measure gas-phase naphthalene from a naphthalene-contaminated aqueous sample.[19] The authors reported detecting a 10-fold lower concentration of naphthalene in the gas phase compared to the aqueous phase, proving that measurement of organic pollutants in the gas phase can increase the sensitivity of microbial biosensors.[16] However, luciferase-based bioluminescent systems are relatively unstable, with physiological differences or inhibitors of energy metabolism reducing light output, while compounds that interfere with fatty acid metabolism or cell membrane synthesis can increase nonspecific light output.[20] Unfortunately, no subsequent study has examined the further use of microbial biosensors to detect gaseous pollutants.

When naphthalene and salicylate (a metabolite of naphthalene) are present, microorganisms (especially *Pseudomonas putida*) utilize naphthalene as a carbon and energy source through the expression of naphthalene catabolic operons, wherein the activated NahR regulatory protein interacts with the promoters of *Pnah* and *Psal* to trigger naphthalene degradation.[21–23] Induction requires the product of only one regulatory gene, *nahR*, which makes NahR a potential biological sensing element for detecting naphthalene and salicylate.

Using this system, we developed a recombinant *Escherichia coli* biosensor for the simple, color-change-based detection of salicylate and naphthalene in the field, and showed that it works in both gas and aqueous phases. Recombinant *E. coli* cells harboring the plasmid pLZNRSAL (encoding NahR and ß-galactosidase) were constructed and suspended (for aqueous-phase detection) or co-immobilized in agarose (for gas-phase detection) with the substrate, chlorophenol red-ß-D-galactopyranoside (CPRG). We then characterized this system and used it to assess salicylate and naphthalene in aqueous-phase, gas-phase, and wastewater experiments.

EXPERIMENTAL

Chemicals

The media components were purchased from Difco (Franklin Lakes, NJ, USA). CPRG and agarose were purchased from Sigma (St. Louis, MO). The genomic DNA isolation kit, plasmid isolation spin kit, and gel extraction kit were purchased from Q-BIO Gene (Santa Ana, CA), Qiagen (Hilden, Germany), and Cosmogenetech (Seoul, Korea), respectively. SacI, XhoI, SalI, HindIII, and T4-DNA ligase were purchased from Takara (Shiga, Japan). The pSV-beta-gal plasmid and pGL3b basic vector were purchased from Promega (Madison, WI). All other reagents were of analytical grade and were used as received without further purification.

Bacteria and Culture Conditions

Escherichia coli DH5α (*hsdR, recA, Thi-1, relA1, gyrA*96) was used to maintain plasmids. *Escherichia coli* DH5α harboring plasmids were cultured at 37°C in TYS media (1% tryptone, 0.5% yeast extract, and 0.5% NaCl) containing 30 $\mu g\,mL^{-1}$ ampicillin. The numbers of colony-forming units (CFUs) were determined by serially diluting the samples and plating the cells on Luria–Bertani agar.

Construction of Plasmid pLZNRSAL

The cloning of *nahR* gene, its own promoter *Pr*, and the promoter of *Psal* from pNRSAL were described previously.[23] The plasmid pNRSAL was digested with SacI and XhoI, and *nahR*/*Pr*/*Psal* fragment (~1.3 kb) was gel isolated. To construct the reporter vector, pGLacZ (~6.6 kb), the *lacZ* fragment (~3.7 kb) of pSV-beta-gal was digested with SalI and HindIII and inserted into pGL3b basic (~2.9 kb) digested with the same enzymes, and then gel isolated to remove the luciferase reporter gene. The *nahR*/*Pr*/*Psal* fragment (~1.3 kb) was introduced upstream of the *lacZ* gene in the pGLacZ vector to generate pLZNRSAL (~7.9 kb) (Figure 1). Plasmids were isolated with a Qiagen spin column kit and transformed into *E. coli*

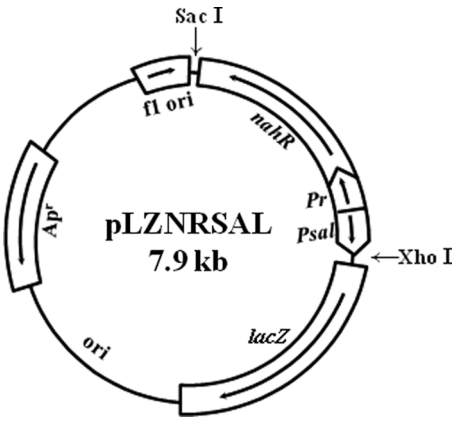

FIGURE 1 Schematics of plasmid pLZNRSAL. The *nahR* gene, its own promoter *Pr*, and the promoter of *Psal* from pNRSAL[15] were digested with SacI and XhoI, and introduced upstream of the *lacZ* (*β*-galactosidase gene) in the pGLacZ vector plasmid.

DH5α by the $CaCl_2$ method. Cloning, ligation, and transformation were carried out using standard methods.[24]

Immobilization of Bacterial Cells

Escherichia coli DH5α cells harboring the plasmid pLZNRSAL were grown for 16 hr at 37°C in 4 mL of TYS medium containing 30 μg mL^{-1} ampicillin, and then mixed (30 μL, approximately 4×10^7 cells/mL) with CPRG (3 m*M*) and melted agarose (final concentration of 0.2%; kept at 42°C). Thereafter, the mixture was quickly dispensed to the wells of a 96-well plate (100 μL/well) and allowed to solidify. The immobilized cells were immediately used for gas-phase measurements or overlain with 40 μL of 50 m*M* KPO_4 buffer (pH 7.0), wrapped in Parafilm, and stored at 4°C until use.

Aqueous-Phase, Gas-Phase, and Optimization Experiments

For aqueous-phase experiments, biosensor cells suspended in the wells of 96-well microplates were incubated for the indicated time periods with various concentrations of ethanol-dissolved aromatic compounds, and *β*-galactosidase activity was detected by the development of a red color (reflecting hydrolysis of CPRG). The results were obtained with the naked eye or by using a microplate reader (Versamax) to assess absorbance at 570 nm.[25] We examined the effects of various cell densities (10–60 μL, approximately $2–6 \times 10^7$ cells/mL) and various concentrations (0.05 μ*M*–10 m*M*) of salicylate and naphthalene on *β*-galactosidase activity, and tested the responses of our biosensor to different aromatic compounds (0.5 m*M* of benzene, toluene, ethylbenzene, *o*-xylene, phenol, and salicylate, and 10 m*M* naphthalene).

To assess naphthalene in the gas phase, co-immobilized biosensor cells with CPRG were exposed to gaseous naphthalene sublimated from the solid form (0.0005, 0.005, 0.05, 0.5, 5 g) in a closed chamber (space volume, 435 mL), and color development was assessed as described earlier. The concentrations of naphthalene in the gas phase can be calculated by the equation[26]:

$$\begin{aligned}&\text{Concentration of naphthalene in gas}(\text{mg/m}^3)\\&= \text{amount of solid naphthalene added in a closed compartment (mg/L)}\\&\bullet\ (\text{molecular weight of naphthalene})/(24.45)\end{aligned} \quad (1)$$

Then, the concentration of gaseous naphthalene (mg/m^3) was converted to molar concentration.

For wastewater assessment, wastewater from Dongseo University and 50 m*M* KPO_4 buffer (pH 7.0) were spiked with salicylate (0.1, 0.5, 1 m*M*) or aqueous naphthalene (1, 5, 10 m*M*) and applied to the aqueous-phase biosensor without any pretreatment. The *β*-galactosidase activities obtained from the wastewater were compared with those obtained from the buffer standards.

RESULTS AND DISCUSSION

Construction of the Biosensor Plasmid pLZNRSAL

In *P. putida*, salicylate induces the expression of naphthalene catabolic operons through the interaction of NahR with the promoters of *Pnah* and *Psal*.[21–23,27] Therefore, the NahR regulatory protein (plus these promoters) could be used to develop a microbial biosensor system for detecting naphthalene and salicylate. The *nahR* gene, its own expression promoter, *Pr*, and the promoter of *Psal* were polymerase chain reaction (PCR) amplified from *P. putida* KCTC 1768, and the resulting PCR product (~1.3 kb *naR*/*Pr*/*Psal* fragment) was introduced upstream of the *lacZ* gene in the pGLacZ expression vector to generate pLZNRSAL (~7.9 kb) (Figure 1). *Escherichia coli* DH5α cells were transformed with pLZNRSAL and suspended with CPRG (for aqueous sensing) or co-immobilized with CPRG in agarose (for gas-phase measurements). In the presence of salicylate or naphthalene, ß-galactosidase hydrolyzes CPRG, leading to the development of a red color that can be visualized with the naked eye or quantitatively assessed as absorbance, using a microplate reader.

Optimum Cell Density and Immobilization

In a microbial biosensor system that uses ß-galactosidase as the reporter, the speed and brightness of color development depend on the density and

growth state of the biosensor cells.[28] Therefore, we assessed the cell density that yielded optimal color development in our system (Figure 2). The response to 0.5 m*M* salicylate (this concentration showed the best response in our sensitivity experiments; see further discussion) reached its highest level with 30 μL of cell suspension (approximately 4×10^7 cells/mL) and plateaued thereafter. Thus, approximately, 4×10^7 cells/mL was used for subsequent experiments.

For the gas-phase experiments, recombinant *E. coli* cells containing pLZNRSAL were co-immobilized with CPRG in agarose to allow the biosensor cells to directly contact the gaseous naphthalene that sublimated from its solid. Previous studies showed that immobilization could protect biosensor cells from harsh environmental conditions, improving their tolerance, operational stability, and long-term use.[28–30] Similarly, we previously reported that agarose-immobilized biosensor cells were stable for 2 weeks at 4°C without any additives or nutrients.[28] We further found that 0.2% agarose was optimum for relatively good color development and firm immobilization, and speculated that a relatively low concentration of agarose with larger pores may allow oxygen and nutrients to diffuse to the embedded microbes.[28] Thus, we co-immobilized the optimum cell density with CPRG in 0.2% agarose for the gas-phase measurements.

Specificity and Sensitivity of the Aqueous System

The specificity of *E. coli* biosensor cells harboring pLZNRSAL was assessed with various aromatic hydrocarbon inducers in the aqueous phase, including BTEX (benzene, toluene, ethylbenzene, and *o*-xylene), phenol, salicylate, and naphthalene (Figure 3). The system did not respond to phenol or BTEX (tested at 0.5 m*M*). Specific responses were obtained for salicylate and naphthalene, but the response to salicylate (tested at

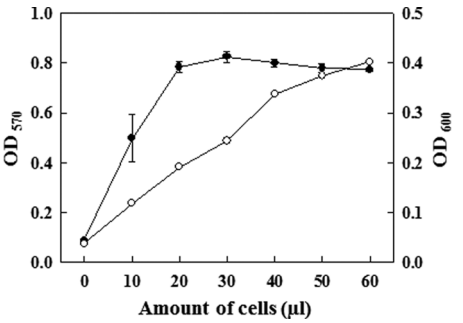

FIGURE 2 Optimum cell density for β-galactosidase activity. Overnight cell cultures were dispensed to 96-well plates, exposed to 0.5 m*M* salicylate and 3 m*M* CPRG for 6 hr, and tested for ß-galactosidase activity (closed circles) by absorbance at OD_{570nm}. The cell biomass (open circles) was measured by absorbance at OD_{600nm}.

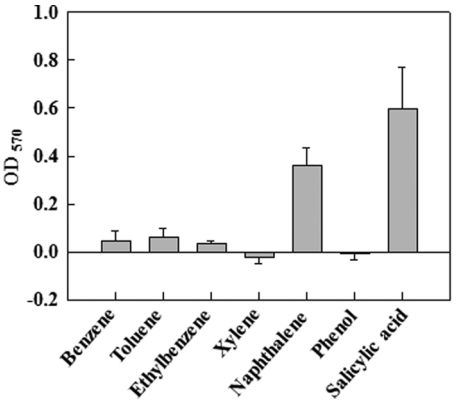

FIGURE 3 Specificity of recombinant *E. coli* biosensor cells containing pLZNRSAL. The biosensor cells were exposed to benzene, toluene, ethylbenzene, *o*-xylene, phenol, and salicylate (all at 0.5 m*M*), and to aqueous naphthalene at 10 m*M*. ß-Galactosidase activities were measured as described in Figure 2, at a cell density of $\sim 4 \times 10^7$ cells/mL.

0.5 m*M*) was more sensitive than that to naphthalene (tested at 10 m*M* to obtain a positive response) (Figure 3). This could be attributed to the low water solubility of naphthalene compared to salicylate, or the sensitivity of the *Psal* promoter used in pLZNRSAL. A previous study showed that the *Psal* promoter is dependent on salicylate, which is a catabolite in naphthalene degradation.[19]

As sensitivity is an important property for microbial biosensors, we further tested the effects of different aqueous concentrations (0.05 µ*M* to 10 m*M*) of salicylate or naphthalene on ß-galactosidase activity (Figure 4). For salicylate, the response was first observed at 0.5 µ*M* (0.069 ppm), peaked at 0.5 m*M* (69 ppm), and decreased thereafter (Figure 4). The apparent lower detection limit of 0.5 µ*M* suggests that this system is slightly less sensitive than optical measurements using luciferase reporter systems, which were previously reported to have a lower detection limit of 0.1 µ*M* for salicylate.[23] This is consistent with previous reports that luciferase reporter systems are generally more sensitive than *β*-galactosidase systems;[4,28,31] this likely reflects that the latter system yields color development without cell lysis, whereas luciferase reporter systems analyze lysed cell extracts. However, the present system has the advantage of detecting color development visible to the naked eye without requiring any instruments or pretreatments. For aqueous naphthalene, we did not obtain any detectable response with 0.05 µ*M*–5 m*M*, and observed only a response at 10 m*M*. This suggests that aqueous naphthalene was not an efficient inducer in this system. The calibration plot for our biosensor cells showed good linearity with regard to salicylate concentrations from 0.5 µ*M* (0.069 ppm) to 10 µ*M* (1.38 ppm; regression curve: $y = 0.26 \log x + 1.48$; $R^2 = 0.98$). We used six consecutive assessments of 0.5 m*M* salicylate to

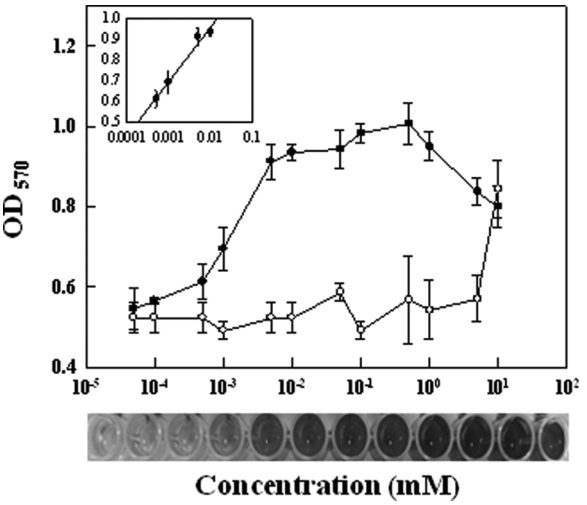

FIGURE 4 β-Galactosidase activities in response to various concentrations of salicylate and aqueous naphthalene. The biosensor cells were treated with 0.05 μM (0.0069 ppm) to 10 mM salicylate (closed circles) and aqueous naphthalene (open circles), and β-galactosidase activities were measured at $OD_{570\,nm}$ or visualized as red color development (at the bottom) to various concentrations of salicylate. The regression curve ($y = 0.26 \log x + 1.48$; $R^2 = 0.98$), which is shown in the inset, indicates linearity between 0.5 μM (0.069 ppm) and 10 μM (1.38 ppm) salicylate.

assess repeatability, and obtained relatively good results (standard deviation, $\pm 2.0\%$, $n = 6$; data not shown).

Assessment of Wastewater

We validated our biosensor cells by testing field samples spiked with salicylate or naphthalene in the aqueous phase (Figure 5). The biosensor cells were exposed to untreated wastewater or buffer (control), both of which had been spiked with salicylate (0.1, 0.5, or 1 mM) or naphthalene

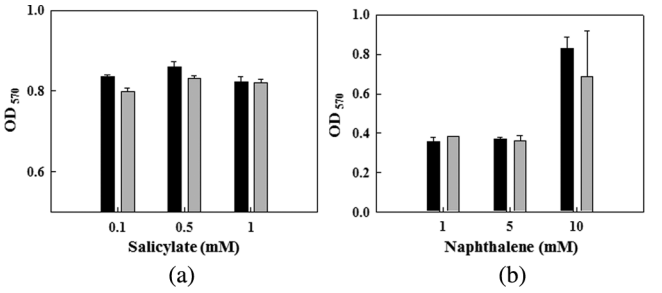

FIGURE 5 Assessment of salicylate (A) and aqueous naphthalene (B) in wastewater or buffer. Biosensor cells were exposed to buffer (black bars) or wastewater (gray bars) spiked with salicylate (0.1, 0.5, 1 mM) or aqueous naphthalene (1, 5, 10 mM), and β-galactosidase activities were measured as described in Figure 2.

(1, 5, or 10 m*M*), and their responses were compared. The biosensor cells showed very similar responses to the spiked wastewater and buffer. The response levels measured in wastewater were slightly lower than the values in buffer, suggesting that chemical impurities in the wastewater might have affected our results. The concentrations of salicylate were estimated based on the regression curve obtained from our biosensor, and the results were approximately consistent with the spiked concentrations (data not shown). This was similar to our previous results.[4,28,31] These results demonstrated that recombinant *E. coli* cells harboring pLZNRSAL could be used for the simple and convenient preliminary detection of salicylate and naphthalene in wastewater, as detected by color change without the need for any sample pretreatment or bulky instruments.

Responses to Gaseous Naphthalene Sublimated From Its Solid

Given that naphthalene is characterized by low water solubility and easy sublimation with significant gas-phase partitioning, we assessed the response of our biosensor cells to gas-phase naphthalene that had sublimated directly from its solid (Figure 6). Biosensor cells co-immobilized with CPRG in agarose were confined with different amounts of solid naphthalene (0.0005, 0.005, 0.05, 0.5, and 5 g) in a sealed chamber, and color development (reflecting the response to sublimated gaseous naphthalene) was measured by absorption at 570 nm at various time points from 0 to 6 hr. Over this period, the responses of the biosensor cells increased slightly in proportion to the amount of solid naphthalene (Figure 6A). When we calculated the concentrations of gaseous naphthalene sublimated from the solid (at 25°C in air) according to Eq. (1), we found that 5 g solid naphthalene in the 435 mL compartment would yield approximately 0.5 m*M*

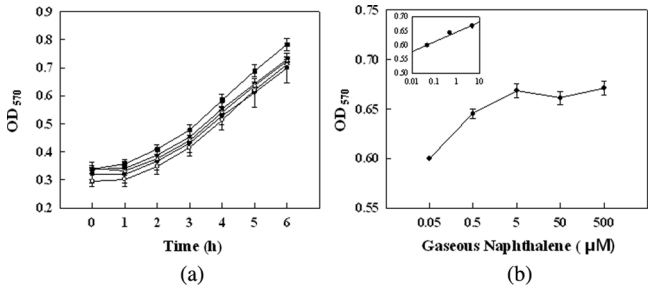

FIGURE 6 Responses to gaseous naphthalene sublimated from the solid at different times (A) and concentrations (B). Biosensor cells co-immobilized with 3 m*M* CPRG in 0.2% agarose were exposed to gaseous naphthalene sublimated from its solid (0.0005, 0.005, 0.05, 0.5, and 5 g from bottom [open circles] to top [closed squares]) in tightly sealed chambers (435 mL). *β*-Galactosidase activity was measured as described in Figure 2. The regression curve ($y = 0.03 \log x + 0.65$; $R^2 = 0.97$), which is shown in the inset, indicates good linearity between 50 n*M* (0.0064 ppm) and 5 μ*M* (0.64 ppm) gaseous naphthalene.

gaseous naphthalene, while 0.0005 g solid naphthalene corresponded to 50 n*M* gaseous naphthalene. Compared with our results from aqueous naphthalene (no response at 5 m*M*), these findings indicated that our biosensor was much more sensitive (about 10^5-fold) to gaseous naphthalene, with a lower limit of detection of 50 n*M* (Figure 6B). These findings are consistent with the previous report that exposure of filter-immobilized *Pseudomonas putida* biosensor cells containing chromosomally inserted *Psal*::*luxAB* to gaseous naphthalene from a naphthalene-contaminated aqueous sample in a closed system exhibited a 10-fold higher luciferase activity than those exposed in solution.[19] Here, we successfully used our *E. coli* biosensor cells (harboring the plasmid pLZNRSAL) co-immobilized with CPRG in agarose for instrument-free, color-change-based monitoring of gaseous naphthalene directly from its solid. Although β-galactosidase systems are less sensitive than luciferase systems, our system showed about 10^5-fold higher sensitivity than those exposed in solution, likely due to the relatively higher copy number of the *lac Z* gene (encoding β-galactosidase) in pLZNRSAL compared to the chromosomally inserted *luxAB* described in the previous report. We also speculate that gaseous naphthalene sublimated from the solid might reach the immobilized cells more quickly than gas arising from the aqueous form. Indeed, the authors of the previous study argued that the diffusive transport rate of naphthalene is approximately 1000-fold higher in gas than in the aqueous phase.[19] The calibration plot for the measurement of gaseous naphthalene by our biosensor cells (Figure 5B) showed relatively good linearity with regard to concentrations from 50 n*M* (0.0064 ppm) to 5 μ*M* (0.64 ppm; regression curve: $y = 0.03 \log x + 0.65$; $R^2 = 0.97$). The result indicates that pollutants could be more effectively detected by monitoring different phases like air. As future tasks, detection of some real gaseous pollutants like chlorinated solvents could be approached by constructing microbial biosensor cells containing corresponding new genetic constructs.

CONCLUSIONS

Recombinant *E. coli* biosensor cells harboring pLZNRSAL were constructed, suspended with CPRG or co-immobilized with CPRG in agarose, and assessed for their ability to detect aqueous salicylate and aqueous or gaseous naphthalene by color development. β-Galactosidase expressed in the presence of salicylate or naphthalene hydrolyzes CPRG, developing a red color that may be observed with the naked eye or measured via absorbance. As expected, the system containing *nahR*::*Psal*::*lacZ* fusion genes was selectively responsive to salicylate and naphthalene, showing a higher sensitivity to salicylate than to naphthalene in the aqueous phase. Although naphthalene, which is characterized by low water solubility, was not an effective inducer in the aqueous system, gaseous naphthalene sublimated

from its solid yielded a far more sensitive (about 10^5-fold) response than aqueous naphthalene. The system was also validated for the detection of salicylate and naphthalene in wastewater. Thus, the recombinant microbial biosensor presented herein could prove useful for monitoring pollutants in the gas and aqueous phases, as reflected by color changes without the need for any pretreatment or bulky instruments.

FUNDING

This work was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (2011-0007702) and partly by a grant (2013) from Innovation Center for Engineering Education of Dongseo University, Republic of Korea. Jae Ho Cho and Da Young Lee contributed equally to this work.

REFERENCES

1. Roy, G.M. *Activated Carbon Applications in the Food and Pharmaceutical Industries*. CRC Press: Boca Raton, FL, 1995.
2. Luch, A. *The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons*. Imperial College Press: London, UK, 2005.
3. Belkin, S. Microbial Whole-Cell Sensing Systems of Environmental Pollutants. *Curr. Opin. Microbiol.* **2003**, *6*, 206–212.
4. Shin, H.J.; Park, H.H.; Lim, W.K. Freeze-Dried Recombinant Bacteria for On-Site Detection of Phenolic Compounds by Color Change. *J. Biotechnol.* **2005**, *119*, 36–43.
5. Keane, A.; Phoenix, P.; Ghoshal, S.; Lau, P.C.K. Exposing Culprit Organic Pollutants: A Review. *J. Microbiol. Methods* **2002**, *49*, 103–119.
6. Shin, H.J. Genetically Engineered Microbial Biosensors for In Situ Monitoring of Environmental Pollution. *Appl. Microbiol. Biotechnol.* **2011**, *89*, 867–877.
7. Dawson, J.J.C.; Iroegbu, C.O.; Maciel, H.; Paton, G.I. Application Of Luminescent Biosensors for Monitoring the Degradation and Toxicity of BTEX Compounds in Soils. *J. Appl. Microbiol.* **2008**, *104*, 141–151.
8. Diplock, E.E.; Mardlin, D.P.; Killham, K.S.; Paton, G.I. Predicting Bioremediation of Hydrocarbons: Laboratory to Field Scale. *Environ. Pollut.* **2009**, *157*, 1831–1840.
9. Paton, G.I.; Reid, B.J.; Semple, K.T. Application of a Luminescence-Based Biosensor for Assessing Naphthalene Biodegradation in Solids From a Manufactured Gas Plant. *Environ. Pollut.* **2009**, *157*, 1643–1648.
10. Wood, K.V.; Gruber, M.G. Transduction in Microbial Biosensors Using Multiplexed Bioluminescence. *Biosens. Bioelectron.* **1996**, *11*, 207–214.
11. D'Souza, S.F. Microbial Biosensors. *Biosens. Bioelectron.* **2001**, *16*, 337–353.
12. Simpson, M.L.; Sayler, G.S.; Applegate, B.M.; Ripp, S.; Nivens, D.E.; Paulus, M.J.; Jellison, G.E. JrBioluminescence-Reporter Integrated Circuits Form Novel Whole-Cell Biosensors. *Trends Biotechnol.* **1998**, *16*, 332–338.
13. Ivnitski, D.; Ihab, A.H.; Plamen, A.; Ebtisam, W. Biosensors for Detection of Pathogenic Bacteria. *Biosens. Bioelectron.* **1999**, *1*, 599–624.
14. 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 Organophophorous Hydrolase. *Biosens. Bioelectron.* **2001**, *16*, 433–437.
15. Park, H.H.; Lee, H.Y.; Lim, W.K.; Shin, H.J. NahR: Effects of Replacement at Asn 169 and Arg 248 on Promoter Binding and Inducer Recognition. *Arch. Biochem. Biophys.* **2005**, *434*, 67–74.

16. Galvão, T.C.; de Lorenzo, V. Transcriptional Regulators à la Carte: Engineering New Effector Specificities in Bacterial Regulatory Proteins. *Curr. Opin. Biotechnol.* **2006**, *73*, 332–338.
17. Medintz, I.L.; Deschamps, J.R. Maltose-Binding Protein: A Versatile Platform for Prototyping Biosensing. *Curr. Opin. Biotechnol.* **2006**, *17*, 17–27.
18. Shin, H.J. Development of Highly-Sensitive Microbial Biosensors by Mutation of the nahR Regulatory Gene. *J. Biotechnol.* **2010**, *150*, 246–250.
19. Werlen, C.; Jaspers, M.C.M.; van der Meer, J.R. Measurement of Biologically Available Naphthalene in Gas and Aqueous Phases by Use of a *Pseudomonas putida* Biosensor. *Appl. Environ. Microbiol.* **2004**, *70*, 43–51.
20. Heitzer, A.; Applegate, B.; Kehrmeyer, S.; Pinkart, H.; Webb, O.F.; Phelps, T.J.; White, D.C.; Sayler, G.S. Physiological Considerations of Environmental Applications of *Lux* Reporter Fusions. *J. Microbiol. Methods* **1998**, *33*, 45–57.
21. van der Meer, J.R.; de Vos, W.M.; Harayama, S.; Zehnder, A.J.B. Molecular Mechanisms of Genetic Adaptation to Xenobiotic Compounds. *Microbiol. Rev.* **1992**, *56*, 677–694.
22. Williams, P.A.; Sayers, J.R. The Evolution of Pathways for Aromatic Hydrocarbon Oxidation in *Pseudomonas*. *Biodegradation* **1994**, *5*, 195–217.
23. Park, H.H.; Lee, H.Y.; Lim, W.K.; Shin, H.J. NahR: Effects of Replacements at Asn 169 and Arg 248 on Promoter Binding and Inducer Recognition. *Arch. Biochem. Biophys.* **2005**, *434*, 67–74.
24. Sambrook, J.; Fritsch, E.F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*. Cold Cold Spring Harbor: Spring Harbor, NY, 2001.
25. Eustice, D.C.; Feldman, P.A.; Colberg-Poley, A.M.; Buckery, R.M.; Neubaue, R.H. A Sensitive Method for the Detection of Beta-Galactosidase Trasfected Mammalian Cells. *Biotehniques* **1991**, *6*, 739–743.
26. Amoore, J.E.; Hautala, E. Odor as an Aid to Chemical Safety: Odor Thresholds Compared With Threshold Limit Values and Volatilities for 214 Industrial Chemicals in Air and Water Dilution. *J. Appl. Toxicol.* **1983**, *3*, 272–290.
27. Schell, M.A. Molecular Biology of the LysR Family of Transcriptional Regulators. *Annu. Rev. Microbiol.* **1993**, *47*, 597–626.
28. Shin, H.J. Agarose-Gel-Immobilized Recombinant Bacterial Biosensors for Simple and Disposable On-Site Detection of Phenolic Compounds. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 1895–1904.
29. Da Silva, S.; Grosjean, L.; Ternan, N.; Mailley, P.; Livache, T.; Cosnier, S. Biotinylated Polypyrrole Films: An Easy Electrochemical Approach for the Reagentless Immobilization of Bacteria on Electrode Surfaces. *Bioelectrochemistry* **2004**, *63*, 297–301.
30. Alvarez, G.S.; Foglia, M.L.; Copello, G.J.; Desimone, M.F.; Diaz, L.E. Effect of Various Parameters on Viability and Growth of Bacteria Immobilized in Sol-Gel-Derived Silica Matrices. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 639–646.
31. Park, S.M.; Park, H.H.; Lim, W.K.; Shin, H.J. A New Variant Activator Involved in the Degradation of Phenolic Compounds From a Strain of *Pseudomonas putida*. *J. Biotechnol.* **2003**, *434*, 67–74.