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Assessment of heavy metal bioavailability in contaminated sediments and soils using green fluorescent protein-based bacterial biosensors

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Nonpathogenic GFP-based bacterial biosensor is applicable in determining the bioavailability of heavy metals in environmental samples.

Abstract

A green fluorescent protein (GFP)-based bacterial biosensor *Escherichia coli* DH5α (pVLCD1) was developed based on the expression of *gfp* under the control of the *cad* promoter and the *cadC* gene of *Staphylococcus aureus* plasmid pI258. DH5α (pVLCD1) mainly responded to Cd(II), Pb(II), and Sb(III), the lowest detectable concentrations being 0.1 nmol L^{-1}, 10 nmol L^{-1}, and 0.1 nmol L^{-1}, respectively, with 2 h exposure. The biosensor was field-tested to measure the relative bioavailability of the heavy metals in contaminated sediments and soil samples. The results showed that the majority of heavy metals remained adsorbed to soil particles: Cd(II)/Pb(II) was only partially available to the biosensor in soil–water extracts. Our results demonstrate that the GFP-based bacterial biosensor is useful and applicable in determining the bioavailability of heavy metals with high sensitivity in contaminated sediment and soil samples and suggests a potential for its inexpensive application in environmentally relevant sample tests.

Keywords: Green fluorescent protein; Bacterial biosensor; Bioavailable heavy metals; Contaminated sediments and soils

1. Introduction

Environmental contamination by heavy metals is a worldwide problem. It is important to aware the possible effects of increasing levels of environmental heavy metals pollution on human health and the environment. Therefore, it is necessary to develop sensitive, effective, and inexpensive methods which can efficiently monitor and determine the presence and amount of hazardous metals in the environment. Traditionally, the environmental risk caused by heavy metal pollution is determined by quantification of total metals after digestion with strong acids by using conventional analytical methods such as atomic absorption spectrometry and ion chromatography. Additionally, before using these analytical methods, environmental samples require laborious treatment to solubilize the metal ions from the solid matrix (i.e. soils or sediments). However, conventional analytical methods are not able to distinguish between available (potentially hazardous) and non-available (potentially non-hazardous) fractions of metals to biological systems. This is of particular interest with respect to solid environments, e.g. soils, because of the great adsorption capability of heavy metals to solid phase (Vanhala and Ahtiainen, 1994). Moreover, the main drawback of chemical methods is the question of the transfer of the results obtained on nonbiological systems to the biological ones.

Because the bioavailability of metals in environmental samples is not a constant value, but varies with changing environmental conditions, it may be a crucial issue in environmental monitoring. To detect the bioavailable fraction of certain metals, several approaches have been followed. Of which,

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one approach is based on the use of bacteria that are genetically engineered so that a measurable signal is produced when the bacteria are in contact with bioavailable metal ions. In sensor bacteria, expression of a reporter gene is controlled by a metal-responsive regulatory element, which usually originates from bacteria that are naturally resistant to a particular heavy metal. The regulatory element can be coupled to a reporter gene through a gene fusion that upon expression produces a readily measurable signal in response to the particular metal. Thus, in the presence of the particular heavy metal, the amount of reporter protein inside the cell increases. Hence, the amount of a given metal was detected by measuring the reporter protein produced by the sensor bacteria.

Several metal-specific bacterial sensors for the detection of bioavailable metals have been developed (Corbisier et al., 1993; Selifonova et al., 1993; Tauriainen et al., 1998; Biran et al., 2000; Ivask et al., 2001). Of which, bacterial biosensors for Cd(II)/Pb(II) have been previously described mostly utilizing reporter genes such as *lacZ*, *lux*, and *luc* in the transcriptional fusion constructs (Tauriainen et al., 1998; Riether et al., 2001; Shetty et al., 2003). Although the colorimetric enzyme assay and bioluminescence have been very successful as a reporter for Cd(II)/Pb(II) detection in their studies, these detection methods require addition of exogenous substrates or cofactors for signal production. The gene for green fluorescent protein (GFP) from the jellyfish *Aequoria Victoria* (Chalfie et al., 1994) is increasingly being used as a reporter gene, although it has not been used extensively as a reporter for measuring biologically relevant concentrations of pollutants. GFP fluorescence is stable and can be monitored non-invasively in living cells. GFP is also an attractive reporter system because it is easy to use and does not require any exogenous substrates or cofactor. The use of GFP as a reporter protein in the bacterial biosensing system therefore can obviate the need for centrifugation, cell lysis, pH adjustment, and subsequently kinetic enzyme activity measurements.

In this work, we describe the construction of a nonpathogenic *Escherichia coli* whole-cell biosensor for the detection of Cd(II), Pb(II), and Sb(III) by employing red-shifted GFP (*rs*-GFP) as a reporter protein. The sensor plasmid is based on the expression of *rs*-GFP under the control of the *cad* promoter and the *cadC* gene of the *cadA* resistance determinant of *Staphylococcus aureus* plasmid pI258 (Nucifora et al., 1989; Tynecka et al., 1981). In the absence of an effector, the expression of *gfp* gene is repressed. Gene expression is induced and fluorescence can be measured in the presence of the effector. Moreover, despite of many different metal biosensors available little attention has been drawn to the use of these biosensors for the analysis of environmental samples. Therefore, the feasibility of the bacterial biosensor for measuring bioavailable metals in environmental samples has not been well tested. To demonstrate the usability of the GFP-based biosensor, we describe the use of the GFP-based biosensor to measure bioavailable fractions of metals in metal-contaminated sediment and agricultural soil samples. The feasibility of using such a strain to analyze the bioavailability of pollutants in the environment is also discussed.

2. Materials and methods

2.1. Chemicals

Unless otherwise stated, all chemicals used were analytical reagent grade or better and were purchased from Sigma–Aldrich (St. Louis, MO, USA). All media and buffer solutions were prepared using deionized distilled water (Barnstead, Dubuque, IA, USA). Restriction endonucleases and T4 DNA ligase were supplied from New England Biolabs (Beverly, MA, USA). The DNA polymerase used in polymerase chain reaction (PCR) was from Qiagen (Qiagen, Hilden, Germany).

2.2. Construction of biosensor plasmid

Recombinant plasmid was constructed as a transcriptional fusion. Plasmid pI258 isolated from *S. aureus* (NCTC 50581; National Collection of Type Cultures, Colindale, London, UK) was used as a template for PCR to generate the 572 base pairs DNA fragment consisting of the promoter/operator of the *cad* operon and *cadC* gene. PCR primers were designed with either *Eco*RI (forward primer) or *Bam*HI (reverse primer) recognition sequence extensions. DNA amplification was carried out in an automated thermal cycler (Eppendorf, Hamburg, Germany). The amplified PCR product was purified using QIAquick PCR purification kit (Qiagen). The purified PCR-amplified DNA fragment was digested with *Eco*RI and *Bam*HI and was purified from an agarose gel by QIAEX II gel extraction kit (Qiagen). Subsequently, the fragment was cloned into the *Eco*RI and *Bam*HI sites of pPROBE-NT′ (Miller et al., 2000). The resulting recombinant plasmid, pVLCD1 (Fig. 1), was transformed into *E. coli* DH5α by the $CaCl_2$ competent cell method.

2.3. Cultivation of bacteria and induction of GFP fluorescence by effectors

A single colony of *E. coli* harboring pVLCD1 was grown overnight in Luria–Bertani (LB) media supplemented with 50 μg mL^{-1} of kanamycin at 37 °C. The overnight culture was diluted 100-fold in fresh LB medium supplemented with 50 μg mL^{-1} kanamycin and incubated at 37 °C in an orbital shaker at 225 rpm until the optical density at 600 nm (OD_{600}) reached 0.6. Various concentrations of Cd(II), Pb(II) or Sb(III) were added to 2-mL aliquots of bacterial cultures. Optical density of cultures at 600 nm and the fluorescent intensity produced by the bacteria were measured. At least three independent experiments were performed for each effector.

2.4. Measurement of GFP fluorescence in culture

The transcriptional activity of the biosensor was estimated by the measurement of the GFP fluorescence of cells grown in LB medium containing a range of different metal ions or time periods. Cell growth was monitored by the measurement of optical density at 600 nm with a spectrophotometer (Eppendorf). The fluorescence of GFP-producing cells that were grown in culture was measured using a VersaFluor Fluorometer that was fitted with a 490 ± 5 nm excitation filter and a 510 ± 5 nm emission filter (Bio-Rad, Hercules, CA, USA). *E. coli* DH5α carrying pPROBE-NT′ without the promoter/operator of the *cad* operon and *cadC* gene was used as the baseline sample to zero the instrument. Raw fluorescence values were expressed in the instrument's arbitrary relative fluorescence units (RFU). The specific fluorescence intensity (SFI)

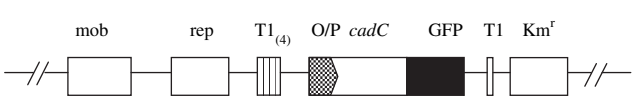

Fig. 1. Schematic organization of the biosensor plasmid pVLCD1. Plasmid harbors genes required for replication (rep) and mobilization (mob). Abbreviation: km^r, gene encoding kanamycin resistance; T1, *Escherichia coli rrnB rRNA* T1 terminator, GFP, green fluorescent protein. The diagram is not drawn to scale.

is defined as the raw fluorescence intensity expressed in RFU divided by the optical density at 600 nm measured at each time point. At least triplicate measurements were obtained for each sample.

2.5. Selectivity studies

The induction of the sensing system by a variety of metal ions, including As(III), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Ni(II), Sn(II), Cd(II), Pb(II), Sb(III), and Zn(II) was studied by measuring the green fluorescence produced. Each metal ion (1 μmol L^{-1}) was added to bacterial sensor culture at a cell density of 0.6 OD_{600}. The cells were incubated for 2 h at 37 °C, and then the specific fluorescence intensity was measured as described above. At least three independent experiments were performed for each kind of metal ion and mixtures of metal ion assays.

2.6. Testing of contaminated sediment and soil samples with bacterial biosensor

Environmental samples were collected from several canals and agricultural lands, known to have heavy metals contamination, from Chunghua County, Taiwan in August of 2003. Before the analysis, the samples were air-dried and sieved to 2 mm. Soil–water extracts were prepared by mixing the air-dried soil with deionized water using the soil–water ratio of 1/9 (w/v). Subsequently, the suspensions were shaken at room temperature for 24 h, followed by centrifugation at 13,000 *g* for 10 min, and then the supernatants (soil–water extracts) were used for chemical and biosensor analyses.

For chemical analysis, concentrations of Cd(II), Pb(II) in water extracts of soil samples were determined with inductively coupled plasma atomic emission spectroscopy analyzer (PerkinElmer 3000SC, Norwalk, CT, USA). Certified standards (PerkinElmer) were run with every determination.

For biosensor assay, the environmental sample was tested by adding 500 μL of soil–water extracts sample to 250 μL of 6× concentrate of LB medium, 10 μL of LB medium, and 740 μL of DH5α cells harboring the pVLCD1 plasmid in LB medium at a cell density of 0.6 OD_{600}. The cells were incubated for 2 h at 37 °C, and then the specific fluorescence intensity was measured using the procedures described above. Samples containing known concentrations of Cd(II) in place of the 10-μL portion of LB medium were tested in parallel with 500 μL of deionized, distilled laboratory water in place of environmental sample to generate a standard curve. Standard curve was derived from linear regression of the average fluorescence value at each particular Cd(II) concentration, and then the concentrations of Cd(II) equivalent in the environmental samples were calculated from the standard curve. In order to examine possible inhibitory effects on fluorescence resulting from chemicals besides the effector compounds in the environmental sample, 10 μmol L^{-1} of Cd(II) was added to the environmental sample, and the green fluorescence emission was compared to that for a positive control containing the same concentration of Cd(II) in deionized water.

2.7. Data analysis

The experiments were performed at least three times for error analyses. The data were used to calculate the standard deviations, represented by error bars in the figures. Student's *t* test analysis at $\alpha = 0.05$ level was performed to check results for significance. Standard curve fits were done by linear regression analysis.

3. Results

3.1. Description of the bacterial biosensor

The *cad* promoter and the *cadC* gene of the *cadA* resistance determinant of *S. aureus* plasmid pI258 was cloned into the broad-host-range vector pPROBE-NT′ (Miller et al., 2000) upstream from the *gfp* gene, creating a P_{cad}-*gfp* transcriptional fusion that was designated pVLCD1 as shown in Fig. 1. When DH5α cells were transformed with pVLCD1 recombinant plasmid, GFP fluorescence was observed in response to Cd(II), Pb(II), Zn(II), and Sb(III) resulting in a statistically significant increase in the fluorescence intensity relative to that of cells with no-effector control. A representative green fluorescence image of the biosensor strain exposed to an effector is shown in Fig. 2.

The selectivity of the bacterial biosensor to metal ions was evaluated. The bacterial cells harboring pVLCD1 plasmid were treated with 1 μmol L^{-1} of various metal ions for 2 h prior to fluorescence measurements as described in Section 2. The levels of fluorescence of the sensing system subjected to these metal ions are also plotted in Fig. 3. In our experimental treatments, a positive response was observed for Cd(II), Pb(II), Sb(III) and Zn(II). No statistically significant change in green fluorescence was observed for As(III), Co(II), Cu(II), Fe(II), Hg(II), Mn(II), Ni(II), Sn(II) compared to the control as shown in Fig. 3.

Since pVLCD1 responds to more than one effector, and since contaminated sites often contain multiple metal species, the response of the bacterial biosensing system to the mixtures of the present metals was investigated. To examine this potentially complex situation, we performed pairwise metal assays with the biosensor. Each metal ion (1 μmol L^{-1}) was

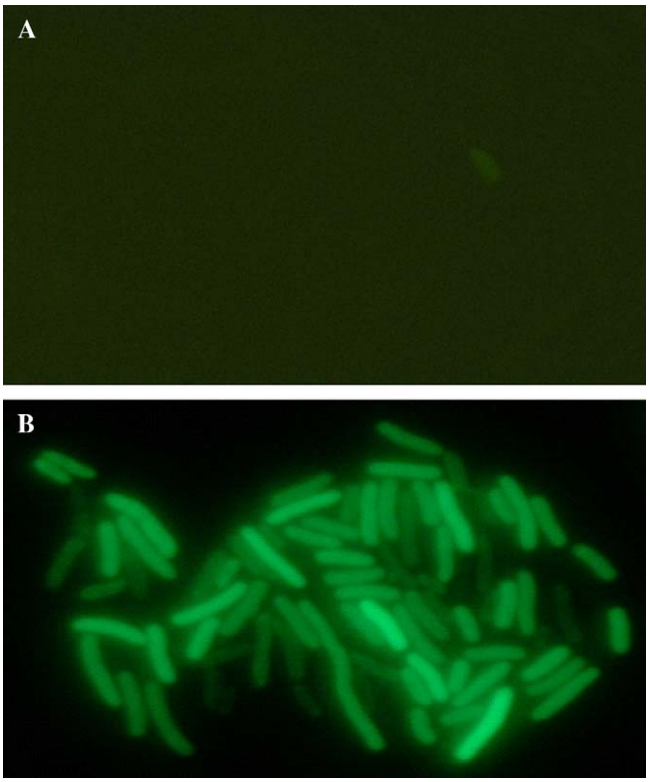

Fig. 2. Fluorescence of biosensor strain carrying pVLCD1 exposed to Sb(III). DH5α (pVLCD1) was treated with 1 μmol L^{-1} of Sb(III) for 2 h at 37 °C in Luria–Bertani (LB) medium, after which green fluorescent protein expression in bacterial cells was visualized by using epifluorescence microscope. Images were captured by using a cooled charge coupled device (CCD) camera. (A) No Sb(III) treatment, (B) bacterial cells treated with Sb(III). Magnification, ×1000.

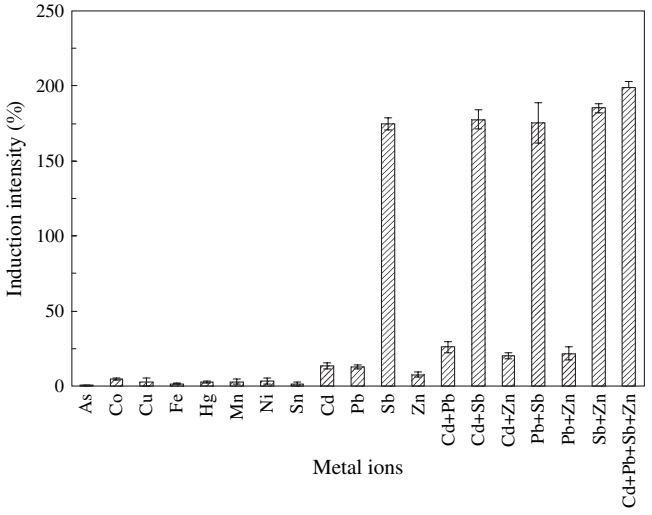

Fig. 3. Selectivity of the bacterial biosensor to metal ions. DH5α (pVLCD1) was treated with 1 μmol L^{-1} of various individual metal ions or mixtures of metal ions for 2 h. Induction intensity (in %) is defined as value of culture specific fluorescence (in SFI) with metal treatment minus culture specific fluorescence (in SFI) of control then divided by culture specific fluorescence (in SFI) of control. Specific fluorescence (in SFI) was measured as described in Section 2. Control refers to no-metal treatment biosensor bacteria. The data presented here are the mean values of at least three independent experiments with the standard deviations.

combined in the same treatment. Additionally, the responses from mixtures of effectors: Cd(II), Pb(II), Zn(II), and Sb(III) were also examined to test whether the individual effector acted in an additive manner. As shown in Fig. 3, these effectors appear to act in an additive manner. The level of green fluorescence for cells treated with other metal combinations other than the aforementioned effector combinations was not significantly different from that of control or effector alone (data not shown).

3.2. Time-dependent induction of green fluorescence with effectors

Time-dependent induction of the bacterial sensor in response to Cd(II), Pb(II), and Sb(III) ion was determined by incubating the cells with metal ions for various time intervals as described in Section 2. Although the biosensor showed response to Zn(II) but to a lesser extent, thus we did not generate time-dependent curve for Zn(II). The induction of green fluorescence of the DH5α (pVLCD1) strain toward the exposure of these metal ions showed a time-dependence (Fig. 4). As shown in Fig. 4, as the time of induction increased, there was an increase in the green fluorescence emitted by the bacterial DH5α (pVLCD1) strain. Interestingly, DH5α (pVLCD1) biosensor readily responds to Sb(III) with the highest induction efficiency. The background fluorescence exhibited by the untreated biosensors did not have any statistically significant fluorescence change during the incubation period (data not shown). The kinetic profile of the biosensor response also showed that during the first 5–6 h of incubation, the

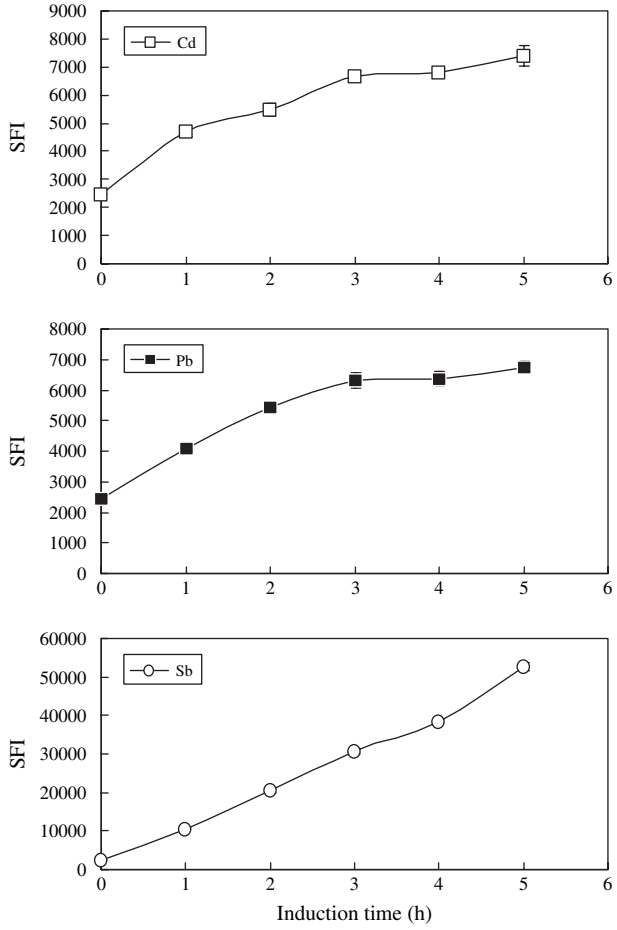

Fig. 4. Time-dependent induction of green fluorescence with effectors. The DH5α cells harboring the pVLCD1 plasmid were exposed to 4 μmol L^{-1} Cd(II), Pb(II), or Sb(III), and the specific fluorescence intensity (in SFI) was determined after different exposure periods. Fluorescence (in SFI) measured with a fluorometer is defined as culture fluorescence divided by culture at a cell density of optical density at 600 nm. The data presented here are the mean values of at least three independent experiments with the standard deviations. The SFI scales for the panels are different.

specific fluorescence intensity continuously increased from the background value (Fig. 4).

3.3. Dose-dependent induction of green fluorescence with effectors

The dose–response relationship of DH5α cells harboring the pVLCD1 plasmid was examined for the effectors: Cd(II), Pb(II), and Sb(III) as described in Section 2. For assay development, a 2-h induction period was chosen since the green fluorescence signal obtained during this time period was sufficiently high enough. Moreover, a 2-h incubation also allows the complete formation of the GFP fluorophore. The fluorescence intensity increased with increasing concentrations of Cd(II), Pb(II), and Sb(III) ions in the sample. Plots of the dose–response relationships of the biosensor to these effectors as measured by fluorometer were shown in Fig. 5. As shown in Fig. 5, the intensity of fluorescence signal emitted increased

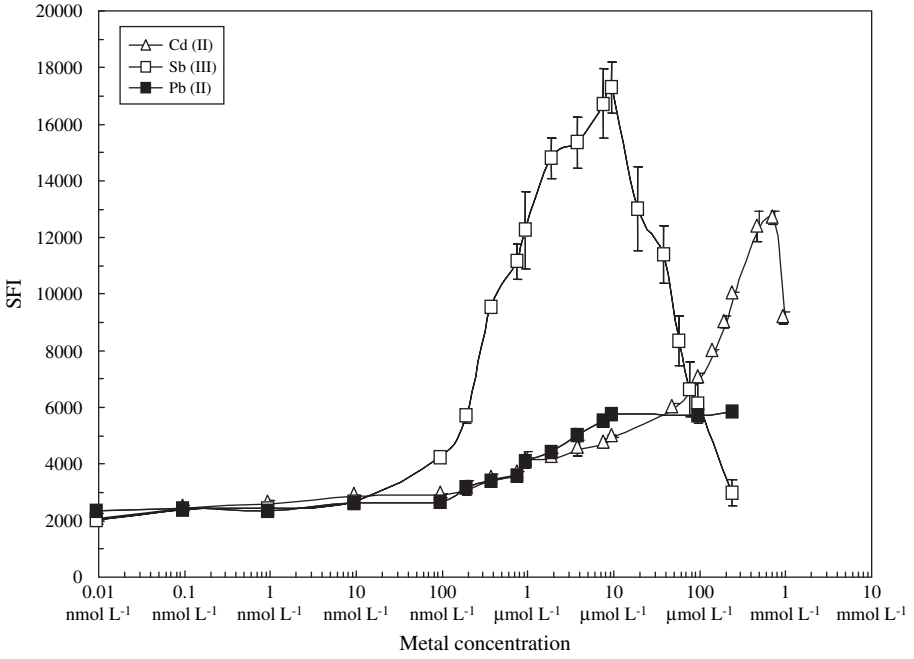

Fig. 5. Dose-dependent induction of green fluorescence by effectors. Fluorescence from DH5α cells harboring the pVLCD1 plasmid was determined after 2-h incubation with various concentrations of metal ions, as described in Section 2. Fluorescence (in specific fluorescence intensity [SFI]) measured with a fluorometer is defined as culture fluorescence divided by culture at a cell density of optical density at 600 nm. The data presented here are the mean values of at least three independent experiments with the standard deviations.

with the concentrations of Cd(II), Pb(II), and Sb(III) to a certain level. At concentrations lower than the detection limit of an effector, binding of *cadC* to the *cad* O/P sequence repressed transcription and translation of *rs*-GFP. Addition of Cd(II), Pb(II), and Sb(III) ions de-repressed *rs*-GFP in the cells.

The detection limits for Cd(II), Pb(II), and Sb(III) observed in our study are well below the metal ions concentrations typically regulated water standard. For Cd(II), 0.1 nmol L^{-1} (0.01 μg L^{-1}) Cd(II) was necessary to induce a statistically significant change ($p < 0.05$) of *gfp* expression. The intensity of green fluorescence increased with increasing amount of Cd(II) to a concentration of 750 μmol L^{-1}, after which the fluorescence started to decrease. This might be due to the toxicity of Cd(II) ions to the bacterial cells. For Sb(III), 0.1 nmol L^{-1} (0.01 μg L^{-1}) Sb(III) was necessary to induce statistically significant change ($p < 0.05$) of *gfp* expression and 10 μmol L^{-1} Sb(III) caused a maximum *gfp* induction. Toxic effect was also noted for Sb(III) at concentration greater than 10 μmol L^{-1}. For Pb(II), the lowest concentration required to induce a statistically significant change ($p < 0.05$) of *gfp* expression was 10 nmol L^{-1} (2.07 μg L^{-1}). At concentration of 10 μmol L^{-1} Pb(II), *gfp* expression was induced to a maximal level. Toxic effect was not observed for Pb(II) at concentrations between 10 and 250 μmol L^{-1}.

3.4. Bioavailability of heavy metals

To demonstrate the utility of this biosensor in measuring actual environmental contamination, sediment and agricultural soil samples with a known contaminant concentration were examined and the results of the biosensor assays were compared to those known concentrations. Sediment and agricultural soil samples were collected from contaminated sites in region of Chunghua County, Taiwan in August of 2003. The bioavailable fraction of the metals was determined from water extract of sediment and soil by using the DH5α (pVLCD1) biosensor. The standard curve was generated with known concentration of Cd(II), and the resulting equation ($y = 1.622x + 2441.9$, $r^2 = 0.9961$) was used to calculate the Cd(II) equivalent concentrations of the samples. By calculating the contaminant concentrations from the standard curve and taking the dilution factor of the assay into account (see Section 2), the final concentrations of contaminants in the samples were shown in Table 1. Because we could not differentiate between possible effectors, the data were expressed as Cd(II) equivalents. Table 1 also compares the results of acid-soluble, water-soluble, and bioavailable fractions of heavy metals in sediment and soil samples by analyzing same batches of environmental samples. Results presented in Table 1 suggest that Cd/Pb contents of the soil samples, as measured by chemical method, are only partially available to *E. coli* DH5α (pVLCD1) biosensor.

Additionally, possible inhibitory effects that might be caused by chemicals besides the effector compounds in the environmental sample were also assessed by spiking the sample with a known concentration of Cd(II) (10 μmol L^{-1}). Subsequently the total SFI was measured and then compared to that for a positive control containing the same concentration in deionized water. No inhibitory effect was detected in this study. Therefore, it is unlikely that the constituents besides the effector compounds in the samples interfered with GFP fluorescence.

Table 1
A comparison of acid-soluble, water-soluble, and bioavailable fractions of heavy metals in sediment and soil samples

Sample	Pb		Cd		
	Acid-soluble ($mg\ kg^{-1}$)[a]	Water-soluble ($\mu g\ L^{-1}$)[b]	Acid-soluble ($mg\ kg^{-1}$)[a]	Water-soluble ($\mu g\ L^{-1}$)[b]	Bioavailable ($\mu g\ L^{-1}$)[c]
W28-1	2786.7	243	12.57	188	392.53 ± 29.15
W30-1	2869.2	684	0.77	ND[d]	425.37 ± 23.69
W32-2	164.7	165	74.83	3650	1054.27 ± 40.80
W43-2	107.7	179	15.83	975	674.83 ± 41.61
S33-1	36.3	183	1.69	ND	ND
S33-3	34.1	184	2.93	ND	ND

[a] From "The field survey and analysis of heavy metals in the sediments of irrigation canals and paddy soils in irrigation area." Report of Agricultural Engineering Research Center, Chungli, Taiwan, ISSN 0255-6081, 2003.
[b] Data were determined by ICP-MS.
[c] Values were calculated by extrapolating green fluorescence emission data to Cd(II)-derived standard curve and were expressed as Cd(II) equivalents.
[d] ND, not detectable.

4. Discussion

Environmental contamination by heavy metals is a worldwide problem. Traditionally, the environmental risk caused by heavy metal pollution is determined by quantification of total metals after digestion with strong acids by chemical analysis. However, these methods are not able to distinguish between available (potentially hazardous) and non-available (potentially non-hazardous) fractions of metals to biological systems. This is of particular interest with respect to solid environments, e.g. soils, because of the great adsorption capability of heavy metals to solid phase (Vanhala and Ahtiainen, 1994). For the determination of the biologically available fraction of heavy metals in solid environments often sequential extraction procedures (Quevauviller et al., 1997), that use different extractants followed by chemical analysis, are applied. The main drawback of these methods is the question of the transfer of the results obtained on nonbiological systems to the biological ones.

In this study we describe the construction and characterization of a GFP whole-cell biosensor for the measurement of biologically available fraction of heavy metals, and we address the feasibility of using GFP as a reporter for pollutant biosensor systems. The sensor plasmid, designated as pVLCD1, is based on the expression of *rs*-GFP under the control of the *cad* promoter and the *cadC* gene of the *cadA* resistance determinant of *S. aureus* plasmid pI258. The use of *gfp* as a reporter gene gives this biosensor the advantages associated with GFP, such as the ability to use fluorescence without the need for exogenous enzyme substrates, the ability to use fluorometry and fluorescence microscopy to monitor gene expression for assessing the bioavailability, and its stability. However, on the other hand, one of the possible drawbacks with measuring GFP in whole cells as it is being produced is that its chromophore forms slowly in the presence of molecular oxygen. As a consequence, any direct measurement is probably an estimate of the total GFP present since a portion of the GFP in whole cells might have yet to become fluorescent.

Induction patterns of DH5α (pVLCD1) slightly differ from those observed in other reports (Yoon et al., 1991; Corbisier et al., 1993; Tauriainen et al., 1998; Shetty et al., 2003). Our results show that robust induction of DH5α (pVLCD1) was observed for Cd(II), Pb(II), and Sb(III), whereas Zn(II) induced *gfp* expression at less extent. Tauriainen et al. (1998) reported that Pb(II) exposure led to a maximum induction as *S. aureus* was used as the host strain. Additionally, Shetty et al. (2003) also observed that Pb(II) was the strongest inducer in their study. In contrast, we found that Sb(III) is the most effective inducer to the DH5α (pVLCD1) biosensor. The discovery of Sb(III) which is an oxyanion as the strongest inducer to *cad* operon is rather unexpected. It has been noticed that *cadC* in the *cad* operon of *S. aureus* plasmid pI258 is considered a member of the ArsR/SmtB family of metalloregulatory repressors (Endo and Silver, 1995). It has been shown that the metalloregulatory α_3N thiolate-rich site in *cadC* exhibits a functional selectivity for larger, softer metal ions like Pb(II), Cd(II), but will accommodate smaller, borderline metal ions like Zn(II) (Busenlehner et al., 2002). Therefore, it is possible that *cadC* binds larger borderline metal such as Sb(III), hence the binding of Sb(III) might mediate allosteric regulation of *cad* O/P binding in vitro. Interestingly, As(III) did not induce fluorescence of strain DH5α (pVLCD1), whereas As(III) and Sb(III) have been found to induce fluorescence of strain DH5α (pVLAS1) (Liao and Ou, 2005), in which the expression of fluorescence was regulated by the *ars* operon and ArsR protein.

Currently the risk assessment of soils and sediments is performed relying on total concentrations of heavy metals obtained using vigorous extraction techniques. In our study, the amount of heavy metal contamination measured by using the DH5α (pVLCD1) biosensor was compared to those measured by using chemical methods. Our results show that the majority of heavy metals remained adsorbed to soil particles: Cd(II)/Pb(II) was only partially available to the bacterial sensor in soil–water extracts. For the induction of the synthesis of the reporter GFP protein the metal should cross the bacterial membrane and enter the cell, which can be considered as a minimum requirement for bioavailability. The whole-cell bacterial sensors, therefore, provide unique bioassay for the detection and measurement of the bioavailable fraction of the metals. Moreover, the induction of the sensors proves that the metals have entered the living cell at low external concentrations.

It should be noticed that quantification of bioavailable metals using the whole-cell bacterial biosensors does not necessarily replace traditional chemical analysis, but whole-cell bacterial sensors can complement analytical chemical methods by distinguishing the bioavailable fraction from the total amount of contaminant in environmental samples. Such information might be highly valuable for risk assessment and for the selection of suitable remediation options. Moreover, bacterial biosensors may reduce the cost of analysis while screening large numbers of samples are required. In addition, comparing metal bioavailability with total metal content from various locations will make it possible to examine the conditions where an inert metal becomes available. These types of data can support bioremediation of metal-contaminated land or sludge.

In addition, the use of bacterial sensors in field conditions is possible. One potential limitation with this method for the testing of environmental samples is that the bacterial sensor used in this study was not perfectly specific to one heavy metal. However, if to analyze a sample in parallel with sensor bacteria responding to different heavy metals, it should be possible to specify the metal causing the response. For example, in this study we analyzed environmental samples in parallel with DH5α (pVLAS1) biosensor that responds to As(III), As(V), and Sb(III) (Liao and Ou, 2005). We found that none of the tested environmental samples induced a significant green fluorescence of strain DH5α (pVLAS1). This indicates that Sb(III) was not present in the tested environmental samples. Moreover, in contaminated sites, there may be many different chemicals besides the inducer compounds present that could be toxic to or interact with *E. coli*, thereby causing inhibitory effects. However, it is impossible to determine the effect of these chemicals, even if the chemicals have been identified by chemical analysis because of the numerous potential combinations of these chemicals. Furthermore, components of the environmental sample such as high salt and high pH values could also inhibit the *gfp* expression of the biosensor. Possible inhibitory effects can be addressed by adding a known amount of an optimal inducer (in this case, Cd(II)) to the unknown samples and then measuring induction differences.

5. Conclusions

In summary, for determining the bioavailable portion of the heavy metals in the sediments and soils, the biosensor bacteria are likely to give a more realistic view. This study demonstrates that the nonpathogenic *E. coli* cells harboring the pVLCD1 plasmid biosensor are useful and applicable in determining the bioavailable fractions of heavy metals in the soil and sediment samples. The biosensor assays are sensitive, fast, inexpensive and less laborious, and thus ideal for screening both presence and bioavailable fractions of heavy metals in the environment. The use of this biosensor may be useful in risk assessment and valuable for the selection of suitable remediation options for contaminated sites.

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