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ORIGINAL PAPER

Specific detection of bioavailable phenanthrene and mercury by bacterium reporters in the red soil

H. Wei · S. Ze-Ling · C. Le-Le · Z. Wen-hui · D. Chuan-Chao

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Abstract Genetically engineered *Pseudomonas putida* reporters (BMB-PL and BMB-ME), which, respectively, carried *phnS-luxCDABE* and *merR-egfp* cassette, were used to determine bioavailable phenanthrene and mercury. Over a spiked range of concentrations and aged for 6 days in red soil samples, the reporters were tested to determine the optimal assay conditions and the bioavailable phenanthrene (0–60 mg kg^{-1}) and Hg^{2+} (0–240 μg kg^{-1}) were evaluated by the signal of the relative fluorescent units and relative luminescence units. Single contamination was carried out and good correlations were obtained between signal strength and pollutant concentrations, whereas interference and bioavailability repression were observed in dual-contamination experiments. Other heavy metal ions at nanomolar level did not interfere with BMB-ME measurement while BMB-PL showed some response to other polycyclic aromatic hydrocarbons or their intermediate products during degradation. Comparing high-performance liquid chromatography methods with the bacterial reporters, both BMB-ME and BMB-PL appeared to have a detection limit (mercury <40 μg kg^{-1}; phenanthrene <24 mg kg^{-1}) similar to the instrumental analysis. Although physical parameters may affect the interaction of pollutants with bioreporter cells, advantages include the inherent biological relevance of the response, rapid response time, and potential for field deployment. Our results strongly suggest that the BMB-ME and BMB-PL bioreporters constitute an adaptable system for easily detecting the bioavailability of mercury and phenanthrene in the red soils.

Keywords Biosensor · Contamination · *Pseudomonas putida* · Red soil

H. Wei · S. Ze-Ling · C. Le-Le · D. Chuan-Chao (✉)
Jiangsu Key Laboratory for Microbes and Functional Genomics, Jiangsu Engineering and Technology Research Center for Industrialization of Microbial Resources, College of Life Science, Nanjing Normal University, Wenyuan Street, Nanjing 210046, Jiangsu, China
e-mail: 13776690639@139.com

Z. Wen-hui
College of Geography Science, Nanjing Normal University, Nanjing 210046, Jiangsu, China

Introduction

Mercury and phenanthrene (PHE) are environmental pollutants of great risk to public health. They have been classified as two of the priority contaminants by the US Environmental Protection Agency (USEPA) due to their potential carcinogenicity, teratogenicity or acute toxicity (Gao and Zhu 2004; Yan et al. 2004; Toyooka and Ibuki 2007). With rapid urbanization and industrialization throughout the world, mercury and PHE are generated by various industrial activities such as mining and smelting of metalliferous ores, electroplating and incomplete combustion of organic matter. As a ubiquitous environmental pollutant (distributed in air, soils and sediments), PHE is often used as a representative indicator of total polycyclic aromatic hydrocarbons (PAHs) contamination (Cebron et al. 2011; Martin et al. 2012). Mercury is also a representative heavy metal (HM) pollutant. Both the presence and risks associated with these compounds in the environment warrant further study.

It is now accepted that bioavailability is a better indicator of risks than total chemical load (Shuttleworth and Cerniglia 1995; Peijnenburg et al. 2004). Measuring bioavailability of mercury and PHE has become essential for calculating their transformation rates, determining toxicity and biodegradability and thereby predicting their

bioaccumulation in different environments (Barkay et al. 1998). However, the bioavailability of pollutants may vary under the influence of mass transfer parameters, which include physicochemical processes governing dissolution, desorption and diffusion, hydrological processes like mixing and, finally, biological processes, such as uptake and metabolism (MacLeod et al. 2001; Johnsen et al. 2005). At the same time, almost all long-term contaminated sites contain more than one pollutant. This raises questions with regard to the differences in bioavailability between single and multiple chemical pollutants. It is important to determine whether one pollutant alters the transfer parameters of another and thereby affect its bioavailability, and how to assess their bioavailability in a convenient way (Stiner and Halverson 2002; Impellitteri et al. 2003).

In recent years, a number of genetically engineered whole-cell bacterial based PAH (include naphthalene fluorene, PHE, anthracene) and Hg^{2+}-specific reporters have been reported and used to detect target chemicals through induced expression of spectroscopically luminescent, chromo- or fluorogenic proteins (Selifonova et al. 1993; Hansen and Sorensen 2000; Kohler et al. 2000; Petanen et al. 2001; Hakkila et al. 2002; Ivask et al. 2002; Petanen and Romantschuk 2002; Belkin 2003; Van der Meer et al. 2004). Even though some of the research focused on the nonspecific detection of pollutants, those reporters possess the ability for semi-quantitative measurements, as obtained with chemical methods, but with inherent biological relevance, for the reporters in principle respond to exactly the fraction of target compound that passes through its cell during the time of the experiment (Van der Meer et al. 2004). Compared with traditional methods like ecotoxicological or cytogenetic assays such as micronuclei test, chromosome aberration and stamen hair mutation assay, the whole-cell bacterial reporters have several advantages in that they are more convenient, effective and specific. However, there seems to be few reports demonstrating biosensors for PHE assessment, and many previous studies focused mainly on contamination by a single pollutant. The whole-cell bacterial reporter method has not received great attention for the simultaneous detection of two or more kinds of pollutants and their bioavailability in one environmental system, especially in soils.

Different pollutants may have different bioavailability in different environmental systems. This is mainly because the bioavailability of pollutants in the environment is influenced by multiple factors such as low aqueous solubility, redox potential, ionic strength, organic matter and difficulties in controlling the physiological activities of the bacterial reporters. This makes it hard to establish a stable relationship between pollutants and their bioavailability (Semple et al. 2003; Benbelkacem et al. 2004; Nadim 2009). In this study, degraded red soil (ultisols and oxisols in US soil taxonomy) was identified as an environmental system polluted by mercury and PHE. Red soil is widespread in the tropical and subtropical areas in the southern part of China, covering about 1.13 million km^2 or 11.8 % of the national land area (Zhong et al. 2007). Owing to the incredible speed of economic and industrial development in the region, red soil has been under tremendous pressure of population growth and environmental pollution. A simple, rapid, and sensitive measurement of pollutants like HMs and PAHs (represented by mercury and PHE) is required. The unique characteristics of red soil such as acerbity, viscidity, leanness, drought and rich aluminiferous ferralsols with low silicon, also provide a unique environment in which to investigate the relationship between red soil and the availability of pollutants.

In this study, a whole-cell bacterium reporter (BMB-PL) was constructed and applied with BMB-ME, created in our previous work, to detect bioavailable PHE and mercury in the red soil of China. The relative luminescent or fluorescent units produced by the reporters were measured and used to establish a stable, specific, and highly sensitive measurement. PHE and Hg^{2+} are analytes with environmental relevance that promised to improve our understanding of PAH and HM bioavailability. Finally, the bioavailable fraction of both PHE and Hg^{2+} during the same experimental time was estimated in contaminated red soil by applying the reporters. Results reported here indicate excellent agreement between the biological and the chemical approach for samples having complex matrices that can exhibit strong effects on bioavailability. At the same time, it was also indicated that the bioavailability of single contaminants is different from that of multiple contaminants.

Materials and methods

Unless otherwise stated, all chemicals used were analytical reagent grade or better and were purchased from Sigma-Aldrich (Shanghai, China). Restriction endonucleases, DNA polymerase, T4 DNA ligase were supplied from TaKaRa Biotechnology Corporation (Dalian, China). DNA primers and fragments were sequenced or synthesized by Genscript Corporation (Nanjing, China). All media and buffer solutions were prepared using distilled de-ionized water (Milli-Q Plus system, Millipore, MA). All plasmids and bacterial strains were stored in our laboratory unless stated.

Samples of top soils (5–15 cm) were collected from the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, located in Yingtan, Jiangxi Province, China (28°15′30″N, 116°55′30″E). Soils of this region are derived from quaternary red clay, dominated by

kaolinite and hydrous mica, and also contain a small amount of vermiculite. The samples were first sieved through a 10 mm and then a 2-mm sieve to remove roots and other vascular material. A detailed chemical analysis was carried out and the results showed that total C, N, P, K and pH were 1.5, 0.10, 0.033, 0.550 % and 6.6, respectively. PHE and Hg^{2+} were not detected by high-performance liquid chromatography (HPLC) in extracts from uncontaminated red soil. All soil samples were sterilized at 121 °C, 30 min before use.

Using a single-step spiking/rehydration procedure (Reid et al. 2000), PHE (methanol solution at 500 mg L^{-1}) or mercuric chloride (water solution 543 μg L^{-1}) was added to soils at a range of concentrations of dry weight soil. Soil samples were mixed thoroughly and left for solvent evaporation for 2 days then adjusted to water content (w/w) at 50 %. The soil samples were supplemented with the desired amount of PHE or Hg^{2+} in two ways: (1) single contamination, PHE or mercuric chloride was added to different soil samples separately; (2) dual contamination, PHE and mercuric chloride were added to the same soil sample. Control soil samples were used throughout to control for the effect of solvent and soil PHE, Hg^{2+} concentrations were confirmed analytically.

A 2.5-kb fragment containing the complete *phnR*/*phnS* promoter region was synthesised by Genscript Corporation (Nanjing, China). This fragment was engineered to have *Bam*HI and *Nco*I restriction sites on both ends and was ligated with a 5.8-kb promoterless bacterial luciferase gene cassette (*luxCDABE*) which was amplified from pMH31 using a pair of primers (L-*Nco*I: 5′-cgaccatggatgactaaaaa-3′ and L-*Bam*HI: 5′-cggggatcctcaactatcaaac-3′). This combined fragment was cloned into a broad host range vector pCM-62 (Marx and Lidstrom 2001) which was obtained from Dr. Shang Guang-dong (Nanjing Normal University). The resultant plasmid pCM-PL carried the *phnR* gene and expressed the presumed regulatory protein which can recognize PHE and form compounds to drive the *phnS* promoter to express the downstream *luxCDABE* cassette, encoding the bacterial luciferase protein (Close et al. 2012). Heat shock (Kim et al. 2003) was then used to transform pCM-PL into a wild-type *Pseudomonas putida* N1 strain which was acquired from China General Microbiological Culture Collection Center (Accession number: 1.1003), and resulted BMB-PL. BMB-ME expresses enhanced green fluorescent protein (EGFP) in response to Hg^{2+} was used as described in the previous work (Wei et al. 2010). *Escherichia coli* DH5α was used for the maintenance of plasmids in all DNA manipulation steps except for the construction of BMB-PL.

Bacterial strains of *P. putida* were cultivated with Luria–Bertani medium (LB, containing 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 10 g l^{-1} NaCl, pH 7.0–7.2) and were grown at 28 °C. Strains carrying reporter plasmids were cultured in LB or on agar plates supplemented with 50 μg mL^{-1} tetracycline. Overnight (ON) cultures were diluted 100-fold into fresh medium supplemented with tetracycline and incubated at 28 °C in an orbital shaker at 225 rpm until the optical density at 600 nm (OD_{600}) reached 0.6.

Filter paper was punched into 5-mm diameter round flakes, soaked in potassium hydroxide containing 5 % potassium hydroxide (w/v) for 10 min, and rinsed with de-ionized distilled water. After drying at 50 °C for 1 h, flakes were then dipped into the buffer solution [33 mM NaH_2PO_4, 33 mM K_2HPO_4, 0.09 mM $(NH_4)_2SO_4$, 50 μg mL^{-1} tetracycline and 5 mM glucose at pH 6.8] for 10 min. After drying at 50 °C again, 5 μL of refreshed BMB-PL ON cultures ($OD_{600} = 0.6$) was added to the surface of the flakes. A second dry flake was then gently placed on top of the first flake to immobilize the BMB-PL reporter system and could then be used directly for the detection of PHE. The prepared double flakes were kept in a petri dish and could be incubated for several days in a cultural incubator with constant temperature at 28 °C and 50 % humidity. The immobilization procedure for BMB-ME was the same, except that tetracycline was not included in the buffer solutions.

The induction assays were carried out in both single and dual-contaminated soil samples prepared as described above. The induction procedures were as follows: 500-mL beakers were prepared and loaded with 200 g red soil samples which were spiked with different concentrations of Hg^{2+} or PHE. Thirty doubled flakes containing immobilized BMB-ME or BMB-PL were distributed evenly onto the leveled soil surface. Another 200 g spiked red soil was added to bury the doubled flakes 5 cm under the surface horizon. The beakers were then transferred into an incubator with constant temperature at 28 °C and 50 % humidity and incubated 1–6 days in the dark. At intervals for the duration of the experiment, 5 doubled flakes buried in the contaminated soil samples were taken out and collected in 2-mL Teflon centrifuge tubes. De-ionized distilled water was added (1.5 mL) to all tubes which were then placed on an orbital mixer (Eppendorf, Thermomixer comfort) and shaken at 400 rpm, 30 °C for 10 min. After centrifugation at 500 × *g* for 1 min, 0.5 mL supernatant was retained and impurities like paper flakes and soil particles were eliminated. Viable cell numbers (colony forming unit, CFU) and the signals produced by the reporters were measured by dilution-plate and luminescence spectrometer Shimadzu RF-540. For fluorescence measurements, samples were excited at 380 nm and measured at 509 nm. The specific fluorescence intensity (SFI) and specific bioluminescence intensity (SLI) are defined as RFU and RLU, respectively, divided by the viable cell

numbers (CFU) measured at each concentration and time point.

A series of orthogonal experiment combinations were also conducted to optimize the conditions of signal inductions. Nine basic orthogonal arrays were tabulated. The notation of each orthogonal array is expressed by its number of rows and letters of columns, as well as the number of levels in each column. The L_9 (3^4) orthogonal arrays, for example, have 9 rows and 4 three-level columns. Based on the meteorological and environmental data of the site where the red soil samples were collected, the concentration of total carbon in the soil (TC: 15, 20, 25 g kg^{-1}), induction time (IT: 1, 3, 6 days), soil water content (WC: 20, 40, 60 %) and temperature (20, 25, 30 °C) were set as four influencing factors on induction response of BMB-ME at 0.1 mg kg^{-1} Hg^{2+} and BMB-PL at 50 mg kg^{-1} PHE. Each factor was set at three levels as 0, 1 and 2. RFU or RLU were measured using the same method as the induction assay.

PHE in soil samples was extracted and cleaned up as previously described (Wang et al. 2010). Water-soluble (WS) Hg^{2+} was extracted from soil samples contaminated by $HgCl_2$ and the analysis of Hg^{2+} in the extractions was carried out by HPLC. Analysis of PHE in the extractions was performed by a HPLC (WATERS 515; USA) fitted with a UV detector and a 4.6 mm × 250 mm reverse-phase C_{18} column using methanol as the mobile phase at a flow rate of 0.65 mL/min (40 °C). Aliquots (20 µL) of each sample were injected into the HPLC system by an auto-sampler. PHE identification was performed by comparison of its retention time with standards and quantification was calculated from the peak area.

All the data were statistically processed using Microsoft Office Excel 2007 and SPSS 13.0. Treatments were replicated three times in the experiments to ensure statistical significance of results (significance was accepted at $P < 0.05$). The images of luminescence and fluorescence expression were acquired using an Olympus BX-41 fluorescence microscope.

Statistical analyses using the general linear model (GLM procedure) in the SAS package were made to identify those variations and/or four-factor interactions that had a significant impact on the signal expression of reporters.

Results and discussion

The pCM-PL plasmid that carried a transcriptional fusion between the phnS putative promoter/operator region and a bacterial luciferase gene cassette (luxCDABE) was successfully constructed (Fig. 1). Bioreporter strain BMB-PL was constructed by transformation of the pCM-PL plasmid into *P. putida* strain N1. Growth characteristics for BMB-PL were the same as for BMB-ME as previously reported (Wei et al. 2010). The strains were shown to express significant fluorescence or bioluminescence in the presence of inducing compounds (Fig. 2).

The influence of four factors (TC, IT, WC and temperature) on fluorescence and bioluminescence expression by the two strains were assessed. Statistical differences in these results were listed in Table 1 and evaluated by analysis of variance. According to the *F* ratio, the order of influence of the four factors D, B, C, A, indicating that temperature may be one of the key factors affecting EGFP expression. Bioluminescence produced by the *lux* gene was mostly affected by the total C in soil, and temperature also a significant influence on the luminescence signal.

Both the highest RFU and RLU were found in experimental trial No. 3 (Table 1). Based on the average RFU and RLU of three experimental trials at each level, the highest levels of signals for different factors are A0, B2, C2, D2, which indicate that EGFP and LUX were best expressed when the TC was 15 g kg^{-1}, the IT was 6 days, the WC was 60 % and the temperature was 30 °C.

Under the optimal assay conditions determined above, the RFU and RLU increased as the soil concentration of Hg^2 and PHE increased from 0 to 240 µg kg^{-1} and 0 to 60 mg kg^{-1}, respectively (Fig. 3). Limits of detection for Hg^{2+} (BMB-ME) and PHE (BMB-PL) were below 40 µg kg^{-1} and 10 mg kg^{-1}, respectively. The good correlations obtained ($R^2 = 0.987$ and 0.994) demonstrates that both BMB-ME and BMB-PL can be used to quantify Hg^{2+} and PHE within a certain concentration range. The luminescent signal decreased rapidly to 0 at 300 µg kg^{-1} (data not shown), probably due to the strong toxic effect of Hg^{2+} ions.

BMB-ME is highly specific to the target Hg^{2+} ions, and most of the other heavy metal ions tested did not interfere with the measurement of Hg^{2+} in the concentration range from 200 nM to 1.2 mM (Wei et al. 2010), whereas BMB-PL was weakly induced by naphthalene, salicylate and

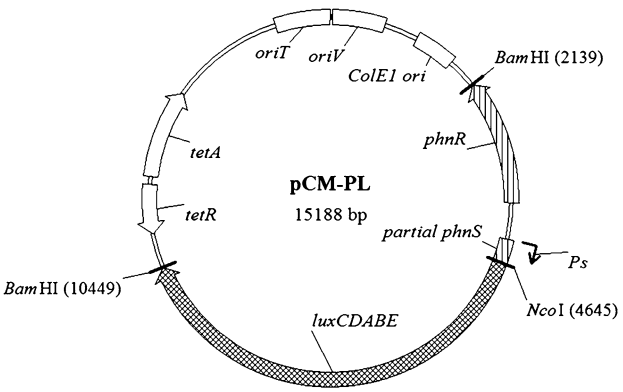

Fig. 1 Structure and restriction map of pCM-PL

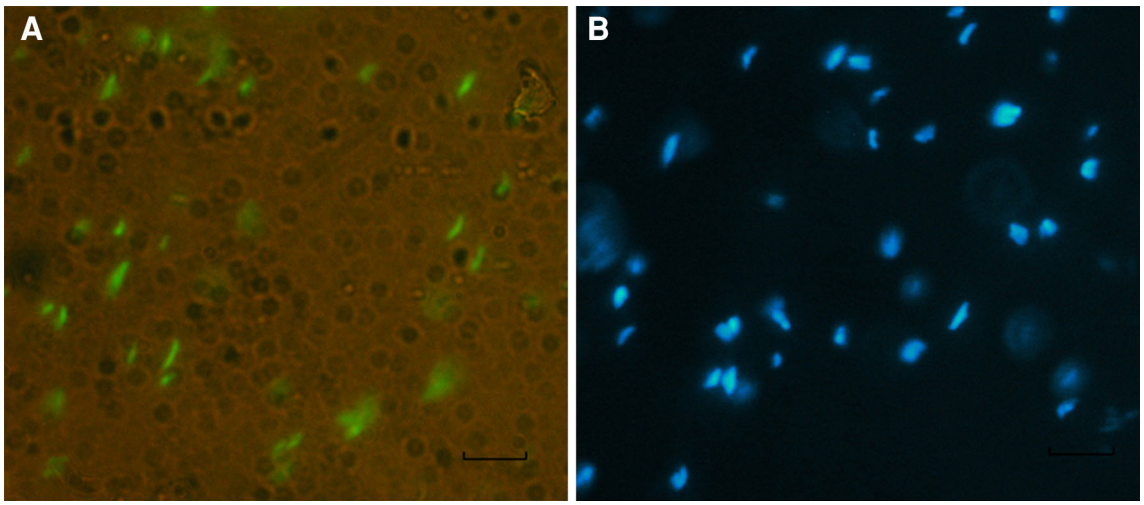

Fig. 2 The induction of BMB-ME and BMB-PL by Hg^{2+} and PHE. **a** BMB-ME fluorescence in visible light. **b** BMB-PL luminescence in the dark. *Bar* 10 μm

Table 1 Layout of the L_9 (3^4) matrix and results for the effect of combined factors on RFU and RLU

BMB-ME						BMB-BL					
No.	A TC	B IT	C WC	D T	Average RFU	No.	A TC	B IT	C WC	D T	Average RLU
1	0	0	0	0	52	1	0	0	0	0	50.6
2	0	1	1	1	113	2	0	1	1	1	113.0
3	0	2	2	2	245	3	0	2	2	2	165.0
4	1	0	1	2	187	4	1	0	1	2	105.0
5	1	1	2	0	66	5	1	1	2	0	98.0
6	1	2	0	1	134	6	1	2	0	1	134.0
7	2	0	2	1	145	7	2	0	2	1	102.5
8	2	1	0	2	205	8	2	1	0	2	58.0
9	2	2	1	0	86	9	2	2	1	0	83.0
K_0	410	384	391	204		K_0	329	258	243	232	
K_1	387	384	386	392		K_1	337	269	301	350	
K_2	436	465	456	637		K_2	244	382	366	328	
F ratio	0.01	0.05	0.03	1.00		*F* ratio	0.68	1.19	0.96	1.00	

K_0, K_1 and K_2 = means of the average RFU or RLU at levels 0, 1 and 2, respectively

TC total carbon, *IT* induction time, *WC* water content, *T* temperature

pyruvate (data not shown). It was assumed that no induction would occur in the presence of other PAHs whose solubility in water is even lower than PHE (0.076 mg L^{-1}) (Tecon et al. 2006).

According to the results of the optimal assay conditions and the environmental site conditions where red soil samples were collected, the dual-contamination assays of PHE and Hg^{2+} were carried out under the following conditions: TC was 15 g kg^{-1}, IT was 6 days, WC was 60 % and the temperature was 30 °C. Spiked with 0–60 mg kg^{-1} PHE and 0–240 μg kg^{-1} Hg^{2+} at the same time, the red soil samples were tested by BMB-PL and BMB-ME simultaneously. Pollutant concentrations and their induction profiles are presented in Fig. 4. The dual-induction curves were different from single induction curves shown in Fig. 1 and the trend lines showed steeper slopes. The induction curve for BMB-ME only changed a little, while significant changes were observed for the BMB-PL induction curve.

Red soil samples spiked with 40–240 μg kg^{-1} $HgCl_2$ were incubated at 30 °C and the concentrations of Hg^{2+}

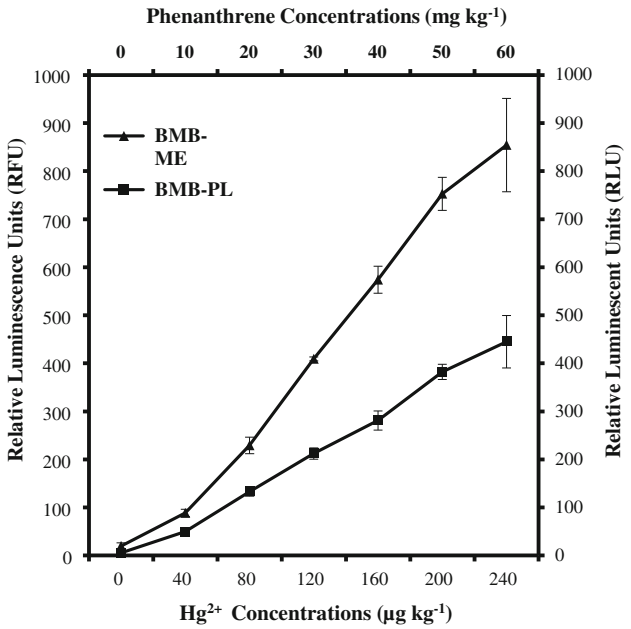

Fig. 3 Induction of BMB-ME and BMB-PL in the presence of single contaminants Hg^{2+} and PHE, respectively. Values represent mean ± standard error ($n = 3$) for fluorescence and bioluminescence

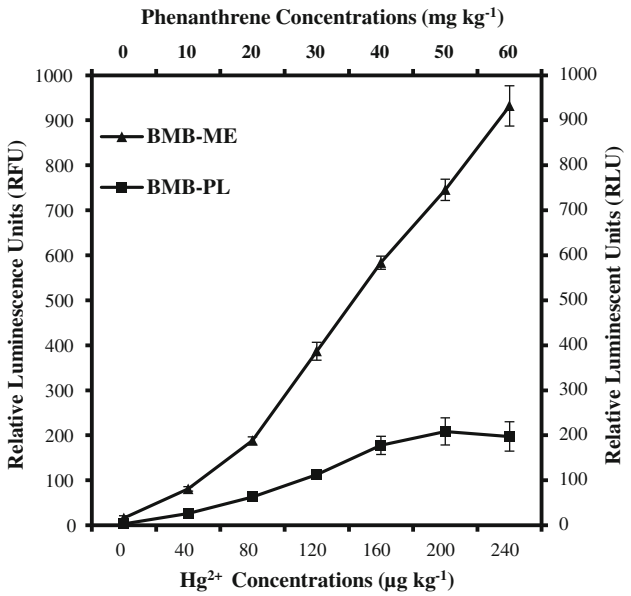

Fig. 4 Induction of BMB-ME and BMB-PL in the presence of both Hg^{2+} and PHE. Values represent mean ± standard error ($n = 3$) for fluorescence and bioluminescence

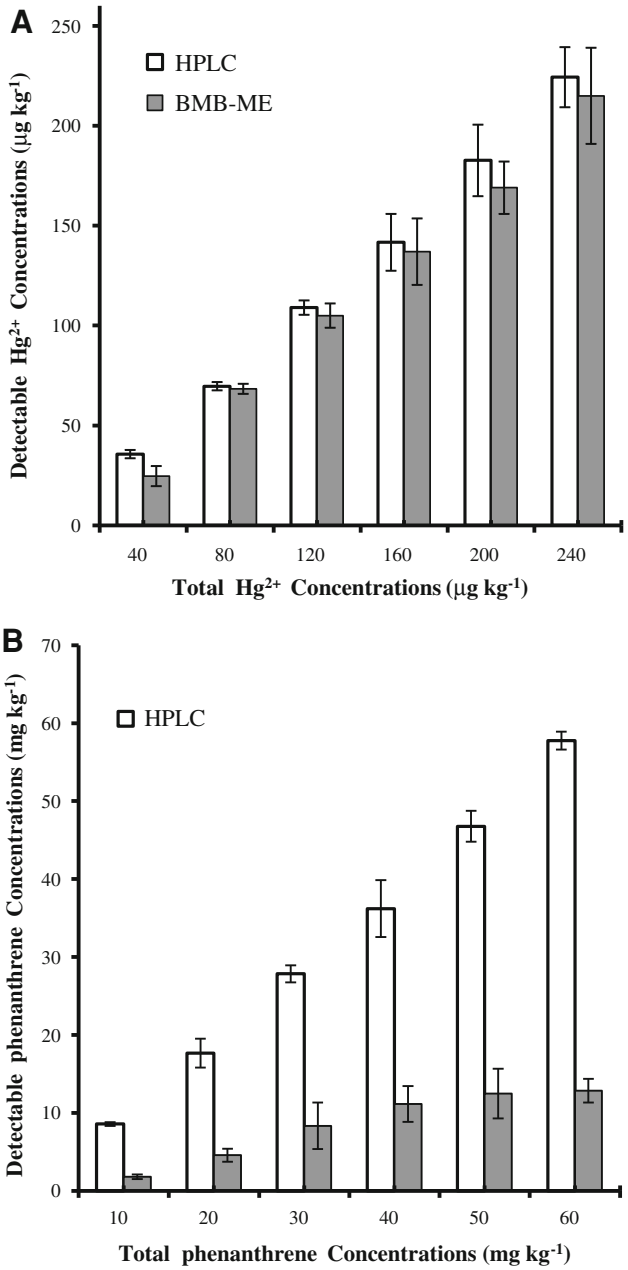

Fig. 5 Comparison between bioreporter and instrumental analysis. **a** Evaluation of Hg^{2+} concentrations by HPLC and BMB-ME method. **b** Evaluation of PHE concentrations by HPLC and BMB-PL method. Values represent mean ± standard error ($n = 3$) for fluorescence and bioluminescence

were measured both by HPLC and the BMB-ME method (Fig. 5a). According to the results, about 10–20 % of the Hg^{2+} was adsorbed in the soil and another 50 % was still in a bioavailable form after 6 days incubation. The total amounts of recovered Hg^{2+} using both methods were similar. This result was not in agreement with previous studies probably due to the different induction time. By comparing the relative percentage with the amount of recovered Hg^{2+}, the reporter strain BMB-ME assay and the HPLC methods performed equally well.

HPLC and BMB-PL were also used to detect PHE spiked in the red soil samples during a 1-week incubation period (Fig. 5b). The initial concentration ranged from 10 to 60 mg kg^{-1}, and it is clear that 80 % of the PHE was detected by HPLC. Although the dose–response of BMB-PL was still quite good, the bioavailable fraction which

was determined by BMB-PL was much less than the total amount of PHE, and this was mainly attributed to the sample extraction procedure used for the HPLC method. Meanwhile, this result suggests that, contrary to the soluble mercury contaminants, PHE is stable in the red soil and becomes relatively unavailable to the microbial biosensor within a certain period of time.

Over the last decade, a great deal of interest has focused on the description and measurement of the bioavailability of contaminants in soils and sediments. However, because of the complexity in soil environmental systems and different forms or types of pollutants, it is difficult to determine the interrelationship among organisms, soils and bioavailability of pollutants as a whole. A number of bacterial whole-cell reporters and soil extraction procedures have been constructed and tested. These methods provide an understanding of the relationships between organisms especially microorganisms and chemical compounds. (Applegate et al. 1998; Willardson et al. 1998; Hakkila et al. 2002; Stiner and Halverson 2002; Casavant et al. 2003; Ivask et al. 2004; Paitan et al. 2004), yet the question of suitable reporter systems for different contaminants has seldom been critically examined and compared, as investigated in this study.

This study described the construction, characterization, and comparison of fluorescence and bioluminescence bacterial reporters for the measurement of bioavailable mercury and PHE in the red soil of China. The recombinant bacterial reporters, designated BMB-ME and BMB-PL, were based on expression of the *gfp* and *luxCDABE* genes, respectively, under the control of the MerR-P_{merT} and PhnR-P_{phnS} promoter. Cells harboring these constructs are capable of producing detectable signals upon exposure to Hg^{2+} and PHE with high sensitivity.

Firstly, on the design of reporter systems, the *lux* gene expression was used to evaluate the bioavailability of PHE. This is mainly because bacterial luminescence has two major advantages for detecting PAHs and other sparingly soluble compounds: a faster response time and higher short-term sensitivities (Leonardi et al. 2008). Both advantages allow a relatively small number of reporter molecules to produce a sufficient and strong signal. In this study, a GFP-marked reporter was also constructed for the PHE testing (data not shown), yet its insensitivity against background values and poor response at low PHE doses made it unsuitable for use. The EGFP signal, on the other hand, is stable for it is independent of enzymatic processes. Once induced by an effector, the green fluorescence keeps accumulating and may be detected for a long period of time even after cell death. This is particularly useful for the detection of highly toxic environmental samples, such as Hg^{2+}.

In addition to the genetic constructs, several factors might have also affected the sensitivities and induction coefficients of these biosensors. These include the host strains, induction times, medium compositions, growth phase of the harvested bacteria, and amounts of bacteria per measurement (Tauriainen et al. 1997; Hansen and Sorensen 2000). In this study, both biosensor systems were produced using the same bacterial strain of *P. putida* N1, and cultivation and measurements were performed identically for both the luminescence and fluorescence bacterial biosensors in the dual-contamination assays. This makes it easy to evaluate the performance of each biosensor under the same conditions. Based on the optimization results, temperature seemed to be the key factor that may affect the EGFP signal. This is due to the conditions for growth of the host strain or the slow folding process of GFP proteins, and it has been demonstrated that GFP requires days at room temperature to reach maximal fluorescence (Baird et al. 2000). The total carbon level in the soil samples governed the expression of LUX signal, meanwhile the reporter system did not function when the host strain was replaced by *E. coli* (data not shown). Since the *P. putida* strain has the ability to degrade a range of aromatic compounds, these phenomena seem to be attributed to the bio-degradation process of PHE and this may help to understand why the BMB-PL was weakly induced by other aromatic compounds or their intermediate products, such as naphthalene, salicylate and pyruvate.

Due to the fact that microorganisms can generally assimilate a hydrophobic organic contaminant which is soluble in the aqueous phase, bioavailability assessment of sparingly soluble compounds, such as PAHs, become problems. A number of studies have investigated the use of various extractions as determinants for bioavailability (Echols et al. 2008; Deepthike et al. 2009). However, these methods seem to be imperfect for the reason that chemical solvent extractions, such as acetone and methanol, may not only interfere with the physiology of bacterial reporters, but also do not reflect the true bioavailability in the environment. Some extraction procedures are also very complex and difficult to perform. Here, soluble Hg^{2+} and sparingly soluble PHE were designated as two typical and representative pollutants. Their in situ bioavailabilities in the red soil of China were evaluated. Without using organic solvent extraction, the different levels of bioavailability indicative of both pollutants were acquired (Figs. 3, 4). It can be seen from the figures, the bioavailability of Hg^{2+} at microgram level is much higher than PHE at milligram level in the red soil of China. It is unknown whether the bioavailability characteristics obtained in this soil are similar to other soil environments, and this also needs to be further investigated.

Pollution of the environment is often caused by more than one kind of contaminant. The interrelationships of different pollutants and their bioavailability have been

investigated in a number of studies (Vrana et al. 2001; Lewis et al. 2004; Rastall et al. 2004; Tusseau-Vuillemin et al. 2007). However, these studies focused mainly on contaminant within the same category, such as HMs or PAHs, and whole-cell bacterial reporters to determine the bioavailability of pollutants have seldom been discussed. In this study, a dual contamination by both heavy metal and polycyclic aromatic hydrocarbon was simultaneously carried out, and the bioavailability of both Hg^{2+} and PHE by each bioreport strain was determined (Figs. 3, 4). Comparing the results of single and dual contaminations, the bioavailability of Hg^{2+} was barely changed by the presence of PHE, whereas the bioavailability of PHE was significantly repressed when Hg^{2+} was also present. The total average bioavailability dropped approximately 11–30.6 % in dual compared to single contaminations. This result may due to the acute toxicity of Hg^{2+}, and may also indicate that the total bioavailability of multiple contaminants was dominated by the pollutant which is more soluble and more available to the bacterial cells (Kong et al. 2011).

Many other instruments or techniques can be employed to detect Hg^{2+} or pollutants in the environment, or to quantify their residuals (Yang et al. 2006). Although other analyses perform well in terms of their accuracy and validity of measurements, they are always focused on the detection of a total amount of contaminants, rather than a direct determination of bioavailability. The whole-cell bacterial reporters in this study, on the other hand, provide a direct relationship between microorganism and Hg^{2+} or PHE. Bioavailability of Hg^{2+} was found to be similar using BMB-ME to the values detected using HPLC within a certain concentration range of Hg^{2+}. BMB-PL, however, was not suitable for detecting the total amount of PHE, yet its merits of low-price, convenience and in situ application in the assessment of bioavailability warrant further investigation. The combined use of both instrumental and bioreporter analysis was also recommended.

Conclusion

In summary, the whole-cell bacterial reporter strains used in this study were able to determine the bioavailable portion of Hg^{2+} and PHE in the red soil of China, providing critical data that can be useful in risk assessment. They also represent a simple, fast, inexpensive, and less-laborious alternative to conventional HPLC measurement. The greatest advantage of this bacterial reporter method may be the ease with which it can be applied for field testing and used for screening both the presence and bioavailable fractions of more than one contaminant in the environment. This is particularly useful when a large number of environmental samples need to be analyzed. On the other hand, many abiotic factors may affect the bioavailability of pollutants and the reporter bacterium in soils, such as clay content, pH, dissolved organic carbon and root exudates (Barkay et al. 1997). The internal concentration of bioavailable contaminants for different reporters may give variable results depending on the ability of the host organism to assimilate and degrade pollutants. Therefore, further studies to examine a variety of environments for host bacteria in order to design more reliable reporters and to investigate other potential host strains for situ application deserve close attention.

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