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Construction of two *lux*-tagged Hg^{2+}-specific biosensors and their luminescence performance

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Abstract Two Hg^{2+}-specific biosensors were constructed using bacterial luciferase as reporter gene and plasmid-free *Pseudomonas putida* X4 and *Enterobacter aerogenes* NTG-01 as host strains. The performance of X4 biosensor was compared with that of NTG-01 biosensor in the same assay conditions. The maximum bioluminescence for X4 (pmerR-luxCDABE-Kan) biosensor was found during the midexponential phase and that for NTG-01 (pmerRluxCDABE-Kan) was at the late exponential phase. The shortest induction time of two biosensors was 30 min. The maximum light signal output for NTG-01 and X4 sensors was observed at the incubation time of 5 and 4 h, respectively. The lowest detectable concentration of mercury by the two biosensors were both of 100 pM at 28°C, pH 7 and an initial cell number of 10^6 CFU ml^{-1}. Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} ions at nanomolar level did not interfere with the measurement by the biosensors. These results show that the sensitivity of the two biosensors is sufficient for the detection of Hg^{2+} under most contaminated environments.

Keywords *LuxCDABE* · Biosensor · Mercury · Luminescence

Introduction

Mercury compounds have been widely used in industry, agriculture, medicine, and so on. Because of their high toxicity and biomagnification in the food chain (Ivask et al. 2002), the detection of mercury in the contaminated environments has been an important issue. Presently, the evaluation of heavy metal contamination in different environments relies mainly on chemical method, which detects only the total concentration of heavy metals and provides limited information on the bioavailability of metals and their potential toxicity to organisms (Farrell et al. 1993).

Biosensor methods have great advantages in that they can measure bioavailability of heavy metals and indicate the degree of biotoxicity for environmental contaminants. The detection of heavy metals in environments by biosensors has received increasing attention. A number of Hg^{2+}-specific biosensors have been reported. The lowest detectable concentration of mercury by *Escherichia coli* pBR28, pOS14, and pOS15 constructed by Selifonova et al. (1993) was 1, 0.5, and 25 nmol L^{-1}, respectively. Hansen and Sørensen (2000) employed *Pseudomonas putida* KT2440: *mer-lux* biosensor to determine the bioavailable mercury in contaminated soil and found that approximately 67 ng of mercury was water extractable from the soil spiked with 2.5 μg $HgCl_2$ g^{-1}. Petänen et al. (2001) demonstrated that the ability of the *Pseudomonas fluorescens*: *mer-lucGR* to quantitatively detect specific mercury was as good as or even better than that of a traditional chemical analysis. Ivask et al. (2002) observed that *E. coli* MC1061 (pmerBR$_{BS}$luc) was not completely specific to mercury and coinducible with cadmium. However, the concentration of Cd^{2+} needed for the induction of MC1061 (pmerBR$_{BS}$luc) was ten times higher than the concentration of Hg^{2+}. Hakkila et al. (2002) compared the bioluminescence performance of different reporter genes, i.e., *lucFF*, *luxCDABE*, *gfp*, and *dsred* in *E. coli* sensors. Their results indicated that the lowest detectable concentration of mercury and the fastest response was found for *lucFF* and *luxCDABE*. Bontidean et al. (2004) proposed that the whole bacterial cell and protein-based

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biosensors gave accurate responses to bioavailable mercury for soil samples, and plant sensors were less useful indicators for mercury-contaminated soil. However, many of the previous studies focused mainly on the detection of bioavailable mercury level under laboratory conditions. Whole bacterial cell biosensor method has not received great attention for practical applications. This is mainly because the physiological activity of the microorganisms is difficult to control. On the other hand, metal bioavailability in soil environment is influenced by multiple factors such as pH, redox potential, ionic strength, organic matter, and clay content.

At present, water, soil, and air have been polluted in some industrial cities in China. Simple, rapid, and sensitive measurements of mercury concentration are urgently required in various fields. The purposes of this study were to construct two biosensors and to compare their bioluminescence performance, specificity, and sensitivity induced by mercury and to test the applicability of the two biosensors for analysis of mercury-polluted soils and to compare with chemical analysis. The further-reaching aim was to develop a method that was suited for the semiquantitative measurement for the bioavailability of mercury in environments. The results indicated that biosensors constructed in this study could be applied to detect mercury contamination in different environments.

Materials and methods

Bacterial strains and plasmids

Bacterial strains and plasmids used in this study are listed in Table 1.

Culture conditions

Luria–Bertain (LB: 10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 10 g l^{-1} of NaCl, pH 7.0–7.2) bacterial strains with sensor plasmid were cultured in LB medium and agar plates supplemented with 100 μg ml^{-1} Ap and 50 μg ml^{-1} Km at 37°C (*E. coil*) or 28°C (*P. putida* and *Enterobacter aerogenes*).

Construction of plasmid pmerRluxCDABE-Kan

Standard recombinant DNA molecular cloning method was used. The *Kan* gene was amplified from pET28-a using the following primers, upper primer: 5'-GTGAATTCTGCGCTC TCCTGTT-3' and lower primer: 5'-GCGAATTCGTATCCG CTCAT-3' (underlined are restriction sites for *Eco*RI). The cycling profile was 30 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 90 s. The size of polymerase chain reaction (PCR) product was 1,400 bp. The PCR fragment and plasmid pmerRluxCDABE were digested with *Eco*RI and purified using DNA Fragment Purification Kit (Tiangen Biotech, Beijing, China). Linear pmerRluxCDABE was treated with calf intestine alkaline phosphatase in order to reduce vector self-linkage and purified. The 1,400-bp Kan fragment was then ligated into linear vector (pmerRluxCDABE) by T4 DNA ligase at 16°C for 8 h, and ligation product was transformed into *E. coli* TG1 cells by $CaCl_2$ method. The correct transformants were chosen by their ability to grow in LB medium supplemented with 100 μg ml^{-1} Ap and 50 μg ml^{-1} Km and to produce luminescence induced by 1 μM $HgCl_2$. The resulting plasmid was extracted and verified for correct structures by *Eco*RI digestion and PCR amplification. Correct transformants were TG1 (pmerRluxCDABE-Kan).

Table 1 Strains and plasmids

Strain or plasmid	Genotype or phenotype	Reference or sources
pmerRluxCDABE	Ap^r, *luxCDABE*	Hakkila et al. (2002)
pET28-a	Km^r	Novagen
pmerRluxCDABE-Kan	Ap^r, Km^r, sensor plasmid for the detection of inorganic mercury	This study
E. coli TG1	*SupE*, *hsdΔ5*, *thiΔ(lac-praAB)* F'[*traD36 praAB*$^+$ *lacI*^{q} *lacZ*ΔM15]	Gibson (1984)
P. putida X4	Plasmid-free derivative of wild-type X4, Ap^r, Co^r, Zn^r, Cd^r, Cu^r	Soil Microbiology Lab, HZAU
E. aerogenes NTG-01	Plasmid-free derivative of wild-type NTG-01, Ap^r, Cd^r, Cu^r	Soil Microbiology Lab, HZAU
E. coli TG1(pmerRluxCDABE-Kan)	TG1 containing sensor plasmid	This study
P. putida X4(pmerRluxCDABE-Kan)	X4 harboring sensor plasmid	This study
E. aerogenes NTG-01 (pmerRluxCDABE-Kan)	NTG-01 including sensor plasmid	This study

Ap^r Ampicillin resistance, *Km^r* kanamycin resistance

Construction of two Hg^{2+}-specific biosensors

The competent cells of X4 and NTG-01 were prepared, and the transforming method for them was based on that developed by Sambrook and Russell (2001) and modified. Bacterial cells were harvested at a suspension OD_{600} value of 0.58. Cells were finally resuspended in 0.1 M ice-cold $CaCl_2$ containing 15% glycerol. The suspension was kept on ice for 30 min before storing at −80°C. For transformation, the mixture of recombinant plasmid DNA and competent cells was heat-shocked at 42°C for 2 min and incubated at 28°C for 2 h before plating. The correct recombinant sensor strains X4 (pmerRluxCDABE-Kan) and NTG-01 (pmerRluxCDABE-Kan) were further used for measurements.

Induced luminescence patterns of biosensors

To ascertain the period at which optimal induction occurred, induced luminescence patterns of biosensors were determined as described by Harkins et al. (2004) and modified in the presence of 2 μM of Hg^{2+}.

Determination of induction time

Biosensors were shaken overnight in LB medium at 28°C. A 500 μl of overnight culture (OD_{600}~3) was added to a 50-ml LB medium with the required antibiotics and cultured to optimum induction phase. Cultures were exposed to 2 μM of $HgCl_2$ and incubated at 28°C. Luminescence was measured every 10 min for the first 2 h and every 1 h for the next 4 h.

Effect of temperature, pH, and initial cell number on Hg^{2+}-induced luminescence

A series of experiments were conducted to evaluate the effects of temperature, pH, and initial cell number on bioluminescence response of two biosensors. Experiments were conducted with (1) different cell numbers (10^8~10^5 CFU ml^{-1}) at fixed pH (7) and temperature (28°C), (2) different temperatures (4°C, 10°C, 24°C, 28°C, 35°C, 37°C, 42°C) at fixed pH (7) and initial cell number (10^6 CFU ml^{-1}), and (3) different pH (3~10) at fixed cell number (10^6 CFU ml^{-1}) and temperature (28°C). $HgCl_2$ was added to obtain a final Hg^{2+} concentration of 2 μM. The cells were incubated for 2 h without shaking before analysis.

Sensitivity and specificity of two biosensors

The sensor strains of X4 and NTG-01 were grown overnight in LB medium. A 500 μl of overnight culture (OD_{600}~3) was added to a 50-ml LB medium with the required antibiotics. The bacteria were harvested during mid and late exponential growth phase. After centrifugation, cells were diluted with fresh LB medium to a suspension OD_{600} value of 0.3. Solutions containing 10 pM to 100 μM of Hg^{2+} ions were added to bacterial suspension and the mixture was incubated at 28°C for 2 h. Bacterial culture was centrifuged at 25°C and suspended in an equivalent volume of phosphate-buffered saline (pH 7.1). A luminometer (DXY-2, Institution of Soil Science, Chinese Academy of Sciences) was used to quantify the bioluminescence produced by 2-ml biosensor cells throughout the whole work, if not otherwise mentioned. Data are presented as the average values of triplicates.

The procedure for specificity measurement was the same as that of the sensitivity. The metal ions used here were Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} at concentrations from 1 pM to 10 mM.

Soil extraction experiments

The contaminated soil was made by adding 25, 50, and 100 μg g^{-1} $HgCl_2$ and incubated for 14 days. One-gram soil was mixed with 10 ml of water and equilibrated at 28°C for 2 h. The suspension was centrifuged at 12,000×*g* for 10 min. A series of tenfold dilutions were made to ensure that mercury concentrations in the extract were within the concentration range of standard Hg^{2+} solution. Two milliliter of soil extract dilution was mixed with 2 ml of biosensor cells (OD_{600}=0.3). After 2 h, the luminescence was measured by luminometer. The amount of water-extractable mercury in soil samples was also analyzed by chemical method.

Results

Construction of two *lux*-tagged Hg^{2+}-specific sensors

The Kan gene was amplified from pET28-a using the specific primers. In order to ensure the specific amplification of the *Kan* gene from pET28-a, the fragment of the *Kan* gene was sequenced by Shanghai Sunny Biotechnology Company (data not shown). Plasmids extracted from correct transformants TG1 (pmerRluxCDABE-Kan) were digested with *Eco*RI and amplified as template with the *Kan*-gene-specific primers. The result showed that the size of PCR product amplified from pmerRluxCDABE-Kan was in agreement with that from pET28-a, which is consistent with the theoretical value, 1.4 kb (data not shown). Therefore, the recombinant plasmid (pmerRluxCDABE-Kan) was successfully constructed.

The recombinant plasmid DNA was transformed into plasmid-free X4 and NTG-01 cells by $CaCl_2$ method. The

result indicated that correct transformants selected in the study contained recombinant plasmid DNA, and no plasmid was found in the cells for the two control strains. The size of plasmids from correct transformants was also correct as compared with control plasmid (data not shown). Therefore, two biosensors were successfully constructed and *luxCDABE* gene was well expressed in their cells by induction of mercury.

Induced luminescence patterns of two biosensors

Bioluminescence throughout growth was measured for two biosensors exposed to 2 μM $HgCl_2$ (Fig. 1). For NTG-01 (pmerRluxCDABE-Kan), bioluminescence increased gradually from 2 to 8 h, peaked at 8 h, dropped at 10 h, and increased slightly at 12 h before declining. For X4 (pmerRluxCDABE-Kan), bioluminescence rose steadily from 2 to 8 h then dropped after maximum bioluminescence was reached at 8 h. These results indicated that two biosensors exhibited basically a similar pattern of induced luminescence. The differences between X4 and NTG-01 sensor were that the optimal induction for X4 biosensor was at the midexponential growth phase, while NTG-01 biosensor achieved maximum bioluminescence during the late exponential phase.

Induction time

Figure 2 shows that the luminescence signal of two biosensors could be detected after 30 min of incubation, increased gradually with induction time, peaked at 4 h for X4 biosensor and 5 h for NTG-01 biosensor, and then declined.

Effect of temperature, pH, and initial cell number on Hg^{2+}-induced luminescence

The sensitivity of the *mer-lux* biosensors as influenced by induction temperature, pH, and initial cell number was investigated. The bioluminescence of two biosensors was increased from nanomolar to picomolar concentration of Hg^{2+} as cell numbers decreased from 10^8 to 10^6 cells per milliliter (Fig. 3). No further improvement was observed for the bioluminescence when decreasing cell density from 10^6 to 10^5 cells per milliliter. Based on these data, the cell density at 10^6 cells per milliliter was assumed to be ideal for the highest sensitivity of luminescence sensors in detecting low concentrations of Hg^{2+}.

Figure 4a illustrates that the bioluminescence of two biosensors increased gradually from 4°C to 35°C, peaked at 35°C, and dropped rapidly at 42°C. Bioluminescence was two to threefold higher for two biosensors at 35°C than that at 28°C. The optimum growth temperature for *E. aerogenes* NTG-01 and *P. putida* X4 is 28°C, which is lower than the temperature of maximum luminescence output.

Figure 4b depicts that luminescence production of X4 biosensor increased gradually from pH 3 to pH 6. The luminescence was stable from pH 6 to pH 8 and declined rapidly at pH 10. NTG-01 biosensors displayed a different pattern as that of X4 biosensor; the luminescence rose gradually from pH 3 to pH 7 then decreased slowly as it reached maximum luminescence at pH 7.

Selectivity and specificity of biosensors

As shown in Fig. 5a, luminescence of X4 biosensor increased with increasing concentration of Hg^{2+} and fell

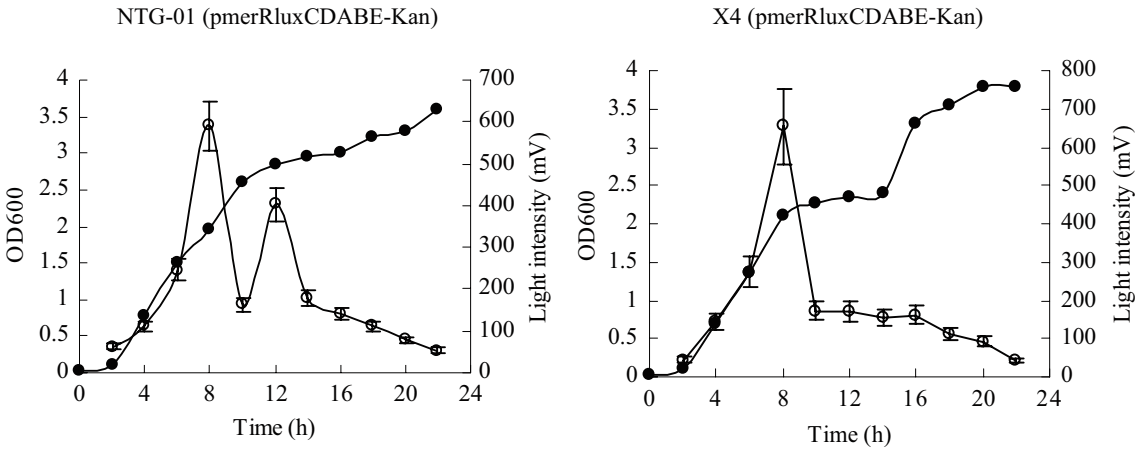

Fig. 1 Growth and bioluminescence of two biosensors induced by 2 μM $HgCl_2$. (*empty circle*) bioluminescence, (*filled circle*) OD_{600}. Data represent mean±standard deviation of three independent measurements

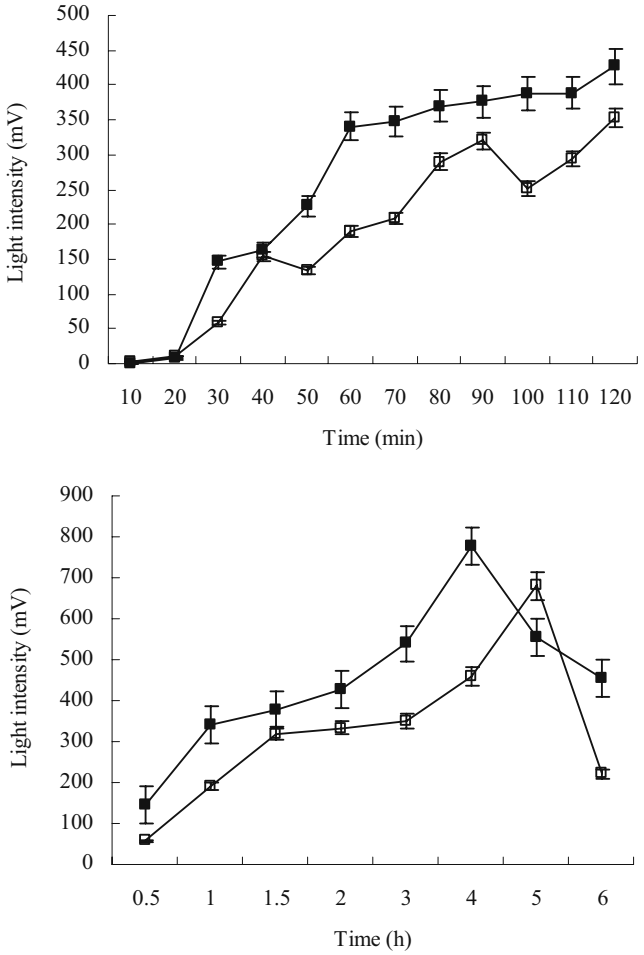

Fig. 2 Induction time course of two strains carrying plasmid pmerR-luxCDABE in the presence of 2 μM mercury. (*filled square*) X4 (pmerRluxCDABE-Kan), (*empty square*) NTG-01 (pmerRluxCDABE-Kan). Data represent mean±standard deviation of three independent measurements

rapidly to 0 at 100 μM of Hg^{2+}; Cd^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, and Cu^{2+} ions induced luminescence at concentrations ranging from 100 nM to 10 mM, and the maximum induction luminescence was 68, 52, 53, 22, and 62, respectively. Specificity of the mercury sensor NTG-01 (pmerRluxCDABE-Kan) was presented in Fig. 5b. In addition to Hg^{2+}, NTG-01 sensor strain was weakly induced by Cd^{2+}, Zn^{2+}, Co^{2+}, Pb^{2+}, and Cu^{2+} ions at concentrations from 100 nM to 10 mM. The maximum luminescence value was 60 with Cd^{2+} ions at 100 μM and 30 with Cu^{2+} ions at 3 mM. These observations suggest that Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} ions do not interfere with the measurement of Hg^{2+} in the concentration range of 100 pM to 100 nM. It is assumed that the two sensors constructed are highly specific to the target Hg^{2+} ions.

The sensitivity of constructed sensors was tested in the same induction condition. Figure 6 demonstrates that the lowest detectable concentration of Hg^{2+} ions by the two biosensors was both 100 pM at the optimal assay conditions. Luminescence of NTG-01 sensor decreased rapidly to 0 at 30 μM, and X4 sensor was 50 μM (data not shown), obviously due to the toxic effect of Hg^{2+} ions.

Quantification of water-extractable mercury in soil

Water-extractable mercury in soil samples was measured by chemical analysis and by using the two biosensor strains. The amount of mercury in soil extraction measured by two biosensors was basically similar. However, the mercury concentrations were lower by bioluminescence than by the traditional method (Table 2). The results showed that only a small fraction of mercury was extracted from the soil.

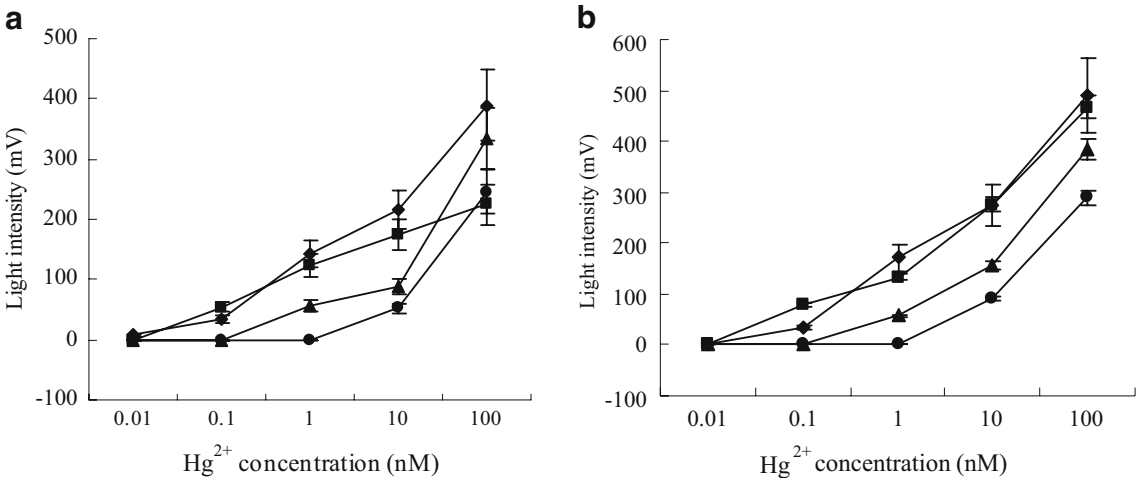

Fig. 3 Effect of initial cell number on biosensors in the presence of Hg^{2+} concentration from 10 pM to 100 nM. **a** X4 (pmerRluxCDABE-Kan), **b** NTG-01 (pmerRluxCDABE-Kan) (*filled square*) 10^5 cells per milliliter, (*filled diamond*) 10^6 cells per milliliter, (*filled triangle*) 10^7 cells per milliliter, (*filled circle*)10^8 cells per milliliter. Data represent mean±standard deviation of three independent measurements

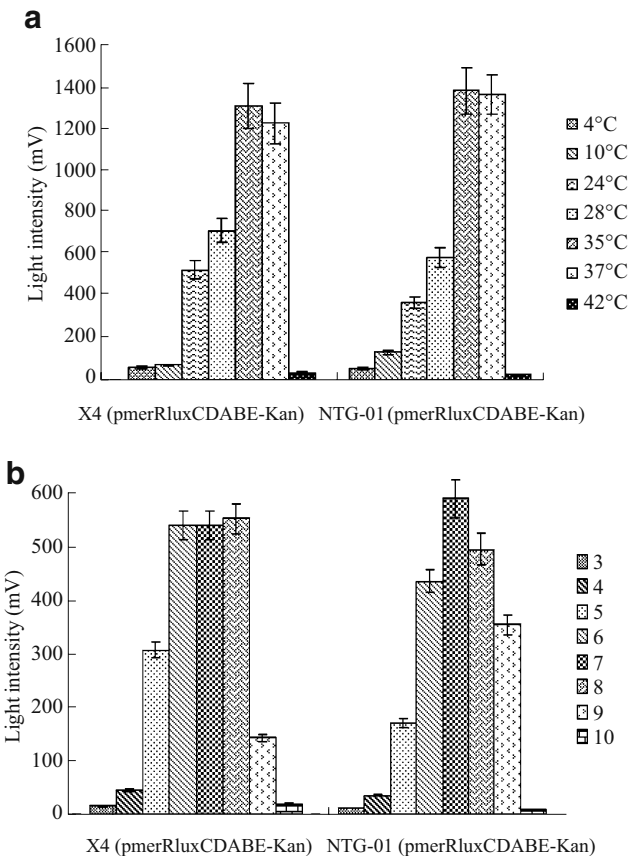

Fig. 4 Effect of incubation temperature and pH on the two Hg^{2+}-specific biosensors in the presence of 2 μM Hg^{2+}. **a** Bioluminescence response of the Hg^{2+} sensor strain X4 (pmerRluxCDABE-Kan) and NTG-01 (pmerRluxCDABE-Kan) at different incubation temperature. **b** Bioluminescence response of strain X4 (pmerRluxCDABE-Kan) and NTG-01 (pmerRluxCDABE-Kan) to Hg^{2+} at different pHs. Data represent mean±standard deviation of three independent measurements

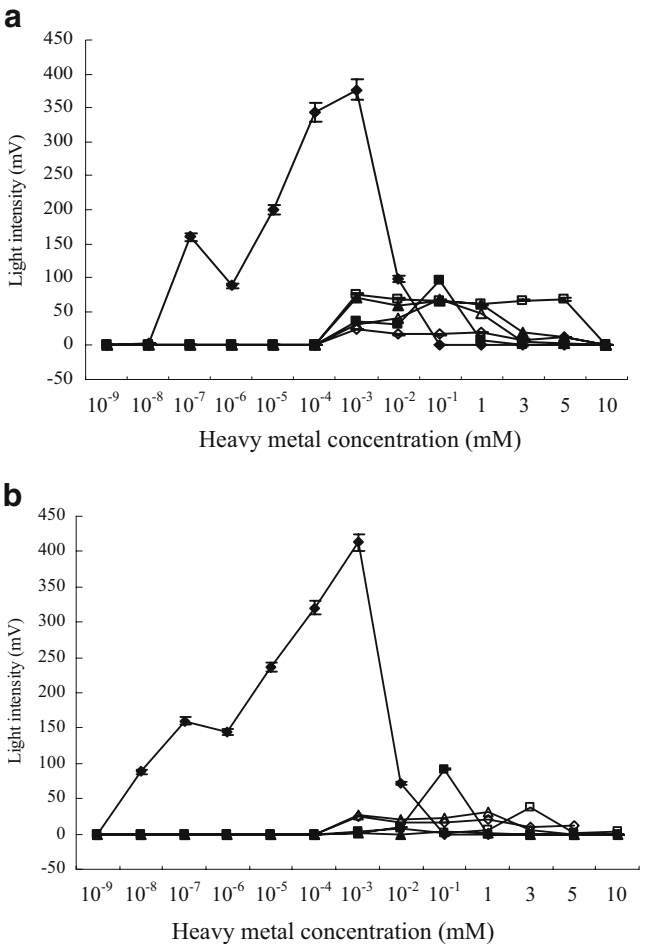

Fig. 5 Specificity of two constructed biosensors in the presence of different heavy metal ions. **a** X4 (pmerRluxCDABE), **b** NTG-01 (pmerRluxCDABE). (*filled squares*) Cd^{2+}, (*filled triangle*) Zn^{2+}, (*empty triangle*) Co^{2+}, (*empty square*) Cu^{2+}, (*empty diamond*) Pb^{2+}, (*filled diamond*) Hg^{2+}. *Error bars* represent the standard deviation of bioluminescence

Discussion

P. putida X4 and *E. aerogenes* were isolated from polluted soil and copper mine area in Hubei Province, respectively. Some characteristics for NTG -01 were reported in detail by Pan et al. (2005). *P. putida* X4 is a Gram-negative bacterium and rod-shaped, and it has capsule and the end flagella. This bacterium could grow in environments with low nutrition and broad range of pH (3–10). More importantly, *P. putida* X4 is resistant to the stress of a variety of heavy metals such as Cd^{2+} (7 mM), Cu^{2+} (4.5 mM), Co^{2+} (7.5 mM), Ag^{+} (0.05 mM), and Zn^{2+} (7.5 mM). The recombinant plasmid containing sensor constructs was transformed to a *P. putida* strain and an *E. aerogenes* strain. *Pseudomonas* was known to be good colonizers of soil (Petänen et al. 2001). Because *P. putida* X4 and *E. aerogenes* NTG-01 have essentially ampicillin resistance, a *Kan* gene was added into the ready-made

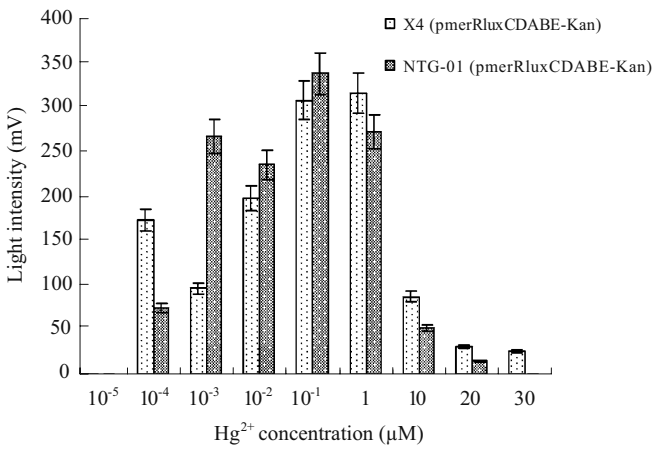

Fig. 6 Dose–response of two biosensors to mercury at different concentrations. Data represent mean±standard deviation of three independent measurements

Table 2 Mercury concentrations in soil extracts measured by luminescent bacterial strains X4 (pmerRluxCDABE-Kan) and NTG-01 (pmerRluxCDABE-Kan) and by chemical methods

Mercury, μg g^{-1}	X4 (pmerRluxCDABE-Kan), μg ml^{-1}	NTG-01 (pmerRluxCDABE-Kan), μg ml^{-1}	Chemical analysis, μg ml^{-1}
25	0.38±0.06	0.28±0.20	0.45
50	0.75±0.16	0.73±0.05	0.98
100	2.31±0.21	2.89±0.26	3.56

One-milliliter soil extract corresponds to 1-g dry weight of soil

plasmid pmerRluxCDABE as screen sign in the study. The host bacteria for Hg^{2+} biosensors have been reported previously for *E. coli*, *P. putida*, *P. fluorescens*, and so on. However, *E. aerogenes* as host bacterium has never been reported to date.

In this work, two whole-cell bacterial biosensor constructs were made by fusing the mercury-inducible promoter, P_{mer}, and its regulatory gene, *merR* with a promoterless reporter gene *luxCDABE*. MerR is a negative regulator of *luxCDABE* gene transcription, binding to the mercury-inducible promoter region in the absence of Hg^{2+}; the expression of the *luxCDABE* gene is depressed. When Hg^{2+} ions are present within the cell, the mercury–MerR complex undergoes a conformational change allowing RNA polymerase to bind with the promoter region; the expression of *luxCDABE* gene is induced and light emission can be quantified.

Induction luminescence patterns of two biosensors constructed in this study are different from those described by Harkins et al. (2004). They found that the optimized induction phase for *E. coli* MC1061 (pmerRluxCDABE) was early exponential. The optimal induction phase for X4 (pmerRluxCDABE-kan) and NTG-01 (pmerRluxCDABE-kan) are mid and late exponential, respectively. These three Hg^{2+} biosensors have the same metal-recognizing protein, the mercury-inducible promoter and reporter gene, but different host bacteria. Different host bacteria may differ in growth and physiological characteristics and show different ability to take up, efflux, and complex mercury ions (Christopher and Raina 2003). Therefore, discrepancies in induction luminescence patterns were observed depending on the bioavailability of Hg^{2+}.

Bioluminescence was detected with living biosensor cell systems, continuous incubation of cells would lead to increased gene expression, and thus absolute luminescence output values are meaningless for metal detection. Bioluminescence values produced by biosensors can only be compared to standards with known metal concentrations under the same conditions (Vandermeer et al. 2003). We observe that a minimum incubation time of biosensor cells with $HgCl_2$ for 30 min was necessary in order to obtain a detectable signal. To insure the accuracy of experiment, 2 h was used as the optimum induction time for the subsequent experiments.

Our present results show that the sensitivity of two biosensors was increased from nanomolar to picomolar concentrations with the decrease of cell number from 10^8 to 10^6 cells per milliliter. The decrease in sensitivity may be ascribed to a reduction in concentration of bioavailable Hg^{2+} ions in the cytoplasm because the whole-cell biosensor was employed to detect internal mercury bioavailability. As we know, MerR is typically a dimer, and its high-affinity mercury binding domain consists of three conserved cysteine residues which forms a highly specific Hg^{2+} coordination site. Both biosensor cell wall and some nonspecific protein including cysteine residues in the cytoplasm may be responsible for the decreasing concentration of Hg^{2+} ions. Similar results were also noted by Pan-Hou et al. (2004) who revealed that the sensitivity of biosensors was improved by decreasing the cell density and the higher cell density in the assay reduced the observed luminescence level, possibly because of metal adsorption by cell wall or light adsorption due to the turbidity of the cell suspension. Rasmussen et al. (1997) proposed that the increase in sensitivity was attributed to a reduction in the number of cellular binding sites that may compete with the regulatory protein, MerR, for the binding of inducer Hg^{2+}. Such sites are composed of negatively charged groups and other ligands which are on the outer surface of the cell or intracellular sequestration with sulfhydryl-containing reducing agents such as glutathione. Therefore, appropriate cell density is required for obtaining high detection sensitivity of mercury at low concentrations of Hg^{2+}.

The bacterial luciferase is present in many kinds of photobacteria. The bacterial luciferases from *Vibrio fischeri* and *Vibrio harveyi* are characteristic of heat instability. They may denature at temperatures higher than 30°C and 37°C, but bacterial luciferases from *Photorhabdus* (*Xenorhabdus*) *luminescens* bacterium are still active at 45°C (Kohler et al. 2000). Bacterial luciferase utilized in this study is from *P. luminescens* (Karp et al. 1998). The two biosensors were found to reach maximum luminescence at 35°C, which is not in accordance with their optimum growth temperature. Therefore, it is assumed that temperature has greater effect on the activity of bacterial luciferase than that of growth and physiological metabolism of sensor strains.

The sensitivity and specificity are two important parameters in evaluating the performance of biosensors. Most of the *mer-lux* biosensors constructed previously have nanomolar sensitivity. This sensitivity is sufficient to detect most of the Hg^{2+}-contaminated and some pristine environmental samples (Pan-Hou et al. 2004). The lowest detectable

concentration of mercury by both of the two biosensors constructed in this study was 100 pM and this sensitivity obtained here exceeded these biosensors (*E. coli* pBR28, pOS14, and pOS15) for mercury described by Selifonova et al. (1993). Moreover, no interference was found with the other heavy metal ions such as Cd^{2+}, Zn^{2+}, Co^{2+}, Cu^{2+}, and Pb^{2+} at nanomolar level. Compared with the measurement by chemical analysis, the biosensors constructed in this study is able to detect mercury-contaminated environments at picomolar to nanomolar levels.

Heavy metal toxicity in the environment is dependent on the concentration of bioavailable metals both internal and external to microbial cells. All whole-cell-specific biosensors reported to date were actually used to measure the internal metal bioavailability; they could not reflect the actual concentration of metallic ion. Mercury ions are usually taken up by specific transporters and not by passive diffusion, but transport pathway is specific for each microorganism. The internal concentration of bioavailable metals for different biosensors may give variable results depending on the ability of the host organism to take up and pump toxic metals out of the cell. Therefore, further studies to examine both uptake and efflux systems for host bacteria in order to design more reliable biosensors and to investigate some other potential host strains deserve close attention.

Conclusions

Two Hg^{2+}-specific biosensors were successfully constructed using bacterial luciferase gene (*luxCDABE*) as reporter and metal-resistant soil bacteria as host organisms. The lowest detectable concentration for mercury by both biosensors was 100 pM at the standard assay conditions. Cadmium, zinc, cobalt, copper, and lead ions (≤100 nM) did not interfere with the measurement. The selectivity and sensitivity of two Hg^{2+} biosensors were adequate for the detection of mercury contaminant ranging from 100 pM to 100 nM in soils and associated environments.

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