Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

JOURNAL OF ENVIRONMENTAL SCIENCES
www.jesc.ac.cn

Application of internal standard method in recombinant luminescent bacteria test

Yong-zhi Wang[1], *Dan Li*[2], *Miao He*[1,*]

1. *Environmental Simulation and Pollution Control (ESPC) State Key Joint Laboratory, School of Environment, Tsinghua University, Beijing 100084, China. E-mail: wyz06@mails.tsinghua.edu.cn*
2. *Fudan University, Department of Environmental Science & Engineering, Shanghai 200433, China*

ARTICLE INFO

Article history:
Received 6 November 2014
Revised 7 March 2015
Accepted 16 March 2015
Available online 26 June 2015

Keywords:
Mercury
Biomonitoring
Recombinant luminescent bacteria
pTHE30–*E. coli*
Internal standard method
False negative

ABSTRACT

Mercury and its organic compounds have been of severe concern worldwide due to their damage to the ecosystem and human health. The development of effective and affordable technology to monitor and signal the presence of bioavailable mercury is an urgent need. The *Mer* gene is a mercury-responsive resistant gene, and a mercury-sensing recombinant luminescent bacterium using the *Mer* gene was constructed in this study. The *mer* operon from marine *Pseudomonas putida* strain SP1 was amplified and fused with prompterless *luxCDABE* in the pUCD615 plasmid within *Escherichia coli* cells, resulting in pTHE30–*E. coli*. The recombinant strain showed high sensitivity and specificity. The detection limit of Hg^{2+} was 5 nmol/L, and distinct luminescence could be detected in 30 min. Cd^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, and Al^{3+} did not interfere with the detection over a range of 10^{-5}–1 mM. Application of recombinant luminescent bacteria testing in environmental samples has been a controversial issue: especially for metal-sensing recombinant strains, false negatives caused by high cytotoxicity are one of the most important issues when applying recombinant luminescent bacteria in biomonitoring of heavy metals. In this study, by establishing an internal standard approach, the false negative problem was overcome; furthermore, the method can also help to estimate the suspected mercury concentration, which ensures high detection sensitivity of bioavailable Hg^{2+}.

Introduction

Mercury is a heavy metal of great risk, and is ranked 6th among the most toxic substances (Nascimento and Chartone-Souza, 2003); it causes both acute toxicity effects and chronic effects by bio-accumulation in living organisms. Major sources of mercury are mining and ore smelting, as well as burning of fossil fuels. According to the UNEP Global Mercury Assessment 2013, mercury released from point sources to water was estimated to be 185 ton/yr, while that from artisanal and small-scale gold mining to land or water was thought to be over 800 ton/yr. To prevent heavy metal pollution of ecosystems and threats to public health, it is important to monitor heavy metal exposure risks. The toxicity of mercury is closely related to its chemical forms (Du et al., 2015). Traditional physicochemical tools are extremely sensitive and specific for pollution quantification, but these methods are not able to distinguish the bioavailable pollution fraction (perceived as potentially hazardous) from the non-bioavailable fraction (Ivask et al., 2002), and they do not

* *Corresponding author*. E-mail: hemiao@tsinghua.edu.cn (Miao He).

http://dx.doi.org/10.1016/j.jes.2015.03.021

appear to be adequate means for automatic online monitoring of heavy metals in the aqueous environment due to their cost, complexity, and pre-treatment requirements.

Mercury resistance has been widely studied for the past six decades (Barkay et al., 2003; Hakkila et al., 2011; Mathema et al., 2011; Moore, 1960). Detoxification of mercury takes place through volatilization and putative entrapment in extracellular polymeric substances (De et al., 2008), and the recognition mechanisms are used in construction of mercury-sensing bioreporters. Recombinant luminescent bacteria are a kind of bioreporter, which can be used to measure bioavailable heavy metals (Hynninen and Virta, 2010) in environmental samples and indicate their biotoxicity. Recombinant luminescent bacteria contain two main functional components (Selifonova et al., 1993): a sensor component for the recognization of the target compound, and a reporter component for the transformation of the target compound concentration into a measurable signal. For the construction of mercury-specific sensing recombinant luminescent bacteria, a system encoded by the *mer* operon is truly specific (Hynninen and Virta, 2010). There are many well-characterized *mer* operons, including Tn21 from *Shigalla flexneri* and Tn501 from *Pseudomonas aeruginosa* (Kholodii et al., 1997). The *mer* operon can be found in both Gram-positive (Bogdanova et al., 2001) and Gram-negative (Iohara et al., 2001) bacteria. Different reporter genes have their own advantages and disadvantages; commonly used genes are firefly luciferase (*luc*), bacterial luciferase (*lux*), and green fluorescent protein (*gfp*). In general, *luxCDABE* is too large and *gfp* has relatively high background signal (Hakkila et al., 2002), while selecting *luc* or *luxAB* as the reporter component requires an additional substance for luminescence (Roda et al., 2001; Yagur-Kroll and Belkin, 2011). No significant luminescence maximum was found between *luc*- and *lux*-based sensors (Harkins et al., 2004). Therefore, *luxCDABE* is frequently used as a reporter gene due to its convenience and sensitivity, in spite of the difficulty of converting the large sequence to a vector or genome. In former works, scientists tended to select *lux* for prokaryote and *luc* for eukaryote host cells, but now the *lux* gene can also be used in eukaryotes (Gupta et al., 2003) or even human cell lines (Close et al., 2010). The first use of *lux* as a bioreporter was done by Enbreghet (Close et al., 2012). In 1987, Rogowsky et al. constructed the pUCD615 plasmid using a promoter-less *luxCDABE* (Rogowsky et al., 1987), and this broad-host-range vector was widely used in recombinant luminescent bacteria. Comparing bioreporters from different research groups, the detection range of *mer*-based bioreporters varies from nmol/L to 10 μmol/L (Harkins et al., 2004).

Although bacterial resistance to heavy metals has been studied for decades, recombinant luminescent bacteria strains for heavy metal monitoring have mostly been used only in laboratory applications (Trang et al., 2005). Application of recombinant luminescent bacteria in practical sample monitoring has faced significant issues, such as parallelism, repeatability, and false positive and false negative problems. The false negative problem is an essential issue for monitoring heavy metals by recombinant luminescent bacteria. Bioluminescence testing might result in a false negative in case of exposure to lethal concentrations of toxic compounds, patchy distribution of nutrients, existence of inhibitory compounds, *etc.* (Sørensen et al., 2006). Several ways to offset this problem have been proposed. In a luminescence constitutive expressing biosensor, the decline of baseline level expression indicated that the bioreporter was inhibited or inactivated (Song et al., 2009). In another work, a bioreporter carrying two distinct reporter genes fused to a constitutively expressed promoter and a stress-inducible promoter was used to detect the genotoxicity and cytotoxicity of environmental samples, where the genotoxic potency was determined by increased bioluminescence of SOS-*Lux* and cytotoxic potency by a decreased GFP-*uv* fluorescence of LAC-*Fluoro* (Baumstark-Khan et al., 2005). In this study, we attempted to apply a simple but useful method to avoid the false negative problem, which is the internal standard method.

In this study, a mercury resistance (*mer*) operon from marine *Pseudomonas putida* strain SP1 was selected (Zhang et al., 2012). This 512 bp element contained the mercury resistance regulator *merR* and its promoter/operator region; detailed sequence data can be found in the GenBank database under the accession number HM217134. The mercury sensing element was used in construction of mercury-sensing recombinant luminescent bacteria; the specificity and sensitivity of the recombinant strain were tested, and a new detection mode to avoid false negative problems is discussed.

1. Materials and methods

1.1. Bacterial strains and growth conditions

Marine *P. putida* strain SP1 was isolated from the Yantai coastal zone in Shandong Province, China (Zhang et al., 2012). *E. coli* RFM443 containing pUCD-recA was laboratory preserved (Huang et al., 2009; Zhang et al., 2007). *E. coli* DH5α chemically competent cells were commercially purchased from TIANGEN Biotech (Catalogue: CB101). *P. putida* was grown at 28°C and *E. coli* DH5α at 37°C in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl). 10 g/L agar was added in LB plates and with 50 μg/mL ampicillin for screening of *E. coli* with recombinant plasmids.

1.2. Construction of pTHE30–E. coli

The *mer* operon of *P. putida* SP1 was amplified using primers as follows: upper primer 5′-CGCGGATCCGCATTTCTCCTTTCGA-3′, and lower primer 5′-CCGGAATTC TTACGCAACGGGAAAT-3′ (underlined are restriction sites for Bam HI and Eco RI). The cycling profile was 30 cycles at 95°C for 45 sec, 58°C for 45 sec and 72°C for 60 sec. The size of the PCR product was about 550 bp, including 512 bp of the mercury resistant gene *merR* and the promoter/operator region of the *mer* gene operon. The PCR fragment was ligated to pGEM-T vector (purchased from Promega, Catalogue: A1360), and the recombinant T-vector was transformed into *E. coli* DH5α cells by the $CaCl_2$ method. Recombinant T-vector and pUCD-*recA* were double-digested by BamHI and EcoRI, then recombined by T4 DNA ligase and transformed to *E. coli* DH5α chemically competent cells. The resulting plasmid *mer*-pUCD615 was verified by sequencing and named pTHE30. Then, pTHE30 was transformed to *E. coli* DH5α, resulting in pTHE30–*E. coli*.

1.3. Luminescence assays

The cultures of pTHE30–*E. coli* were harvested in the exponential phase and diluted with fresh LB medium for testing. Luminescence was measured with a microplate reader (SpectraMax M5) and expressed as relative luminescence units (RLU). Testing samples were added to a 96-well plate with a total volume of 200 μL, including 100 μL heavy metal solution and 100 μL cell suspension. The luminescence of samples was determined for 60 min at 5 min intervals. OD600 values were measured by M5 with 200 μL of cell suspension. The sensitivity and specificity of the recombinant strain were tested with a concentration gradient of mercury and other metal solutions. All measurements were conducted in duplicate.

2. Results and discussions

2.1. Construction of pTHE30–E. coli

The construction of pTHE30–*E. coli* was performed to obtain a newly sensitive bioreporter for mercury monitoring with high specificity and limited response to other heavy metals. For this purpose, a domestic *mer* operon was obtained from marine *P. putida* SP1, previously isolated from the Yantai coastal zone in Shandong Province, China (Zhang et al., 2012). The *mer* operon was subcloned between the BamHI and EcoRI site of pUCD-*recA*, resulting in replacement of the *recA* operon with the *mer* operon. Amp^r and Kan^r in pUCD were helpful for strain screening and maintenance of the plasmid during passages. The recombinant pTHE30 plasmid was transformed to *E. coli* DH5α chemically competent cells, resulting in pTHE30–*E. coli* (Fig. 1), and the *lux* gene was successfully expressed in the presence of mercury.

2.2. Sensitivity and specificity of pTHE30–E. coli

The pTHE30–*E. coli* strain was tested with $HgCl_2$, $CdCl_2$, $CuCl_2$, $ZnSO_4$, $CaCl_2$, $Pb(NO_3)_2$, $MgCl_2$, $MnSO_4$, and $Al_2(SO_4)_3$ in the range 10^{-5}–1 mmol/L. The OD600 value of the cell culture was 0.451, and the cell suspension was prepared by 10 fold dilution with LB.

The recombinant strain showed high sensitivity to Hg^{2+} (Fig. 2), and could respond to Hg^{2+} ranging from 5 to 10 μmol/L. Lower concentrations of analyte (in this case, referring to Hg^{2+}) were not bioavailable to the detection chain after loss by adsorption, evaporation, precipitation, or complexation (van der Meer et al., 2004). However, the total *lux* luciferase activity decreased because higher doses of the analyte became cytotoxic and destroyed the host cells (Escher and Leusch, 2012). In this research, the recombinant train showed no luminescence activity when exposed to 10 μmol/L or higher doses of Hg^{2+}. The minimum exposure time was 30 min; for shorter times, the luminescence of lower Hg^{2+} concentrations was not detectable.

Definition of the lowest observed effective concentration (LOEC) has varied in different publications (Hakkila et al., 2004; Hynninen and Virta, 2010; Hynninen et al., 2010). LOEC was not a subject in this research; the 5 nmol/L value was acquired by visual inspection from the dose–response curve and luminescence after 30 min exposure time and was about 2 times the background signal (Hynninen and Virta, 2010). The testing medium in this research was completely LB (primitive cell culture or LB diluted sample). Medium composition might affect mercury bioavailability for a bacterial strain, and reduce the sensitivity of the luminescence test, thus changing to an appropriate medium would be helpful to achieve a lower detection limit (Barkay et al., 1997; Endo et al., 2003; Rasmussen et al., 2000; Thouand et al., 2003).

When exposed to Cd^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, and Al^{3+} in the range 10^{-5}–1 mmol/L, the recombinant strain did not show a significant light increase. All RLU values during the luminescence test were lower than 100 (Fig. 3), and the strain showed high specificity for mercury detection.

2.3. Use of internal standard to avoid false negative results

The internal standard method is a well-developed method in liquid scintillation counting. In the internal standard method, a certain amount of pure substance is added to samples under analysis as an internal standard substance. For this research, 100 nmol/L Hg^{2+} was added to analyzed samples of different heavy metal concentrations. After an exposure time of 60 min,

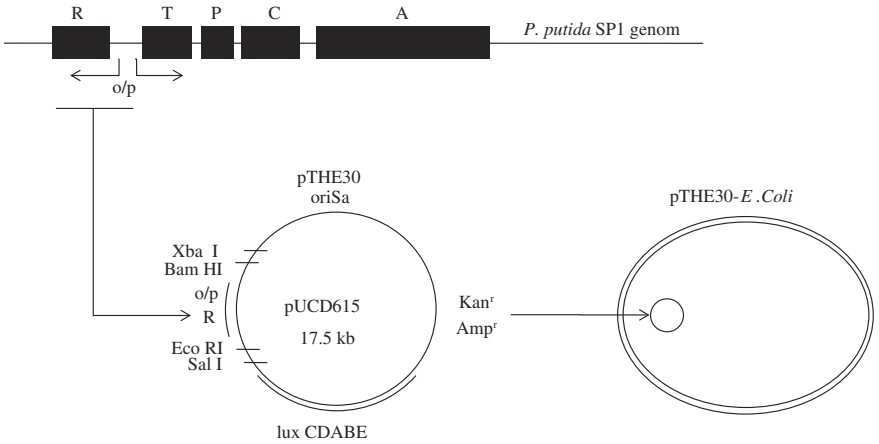

Fig. 1 – Construction of pTHE30–*E. coli*. Gene fragment of *mer* gene. R: mercury regulatory protein gene and o/p: the promoter/operator sequence from *P. putida* SP1 were fused upstream of promoter-less *lux* gene in pUCD615, then transformed to *E. coli* DH5α.

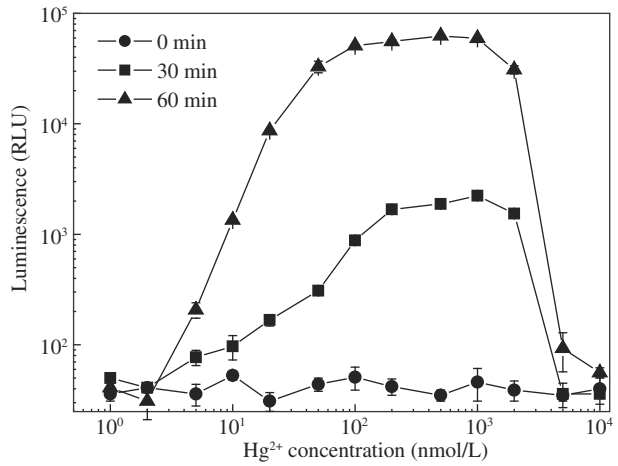

Fig. 2 – Luminescence (relative luminescence unit, RLU) of pTHE30–*E. coli* at different time after addition of Hg^{2+}.

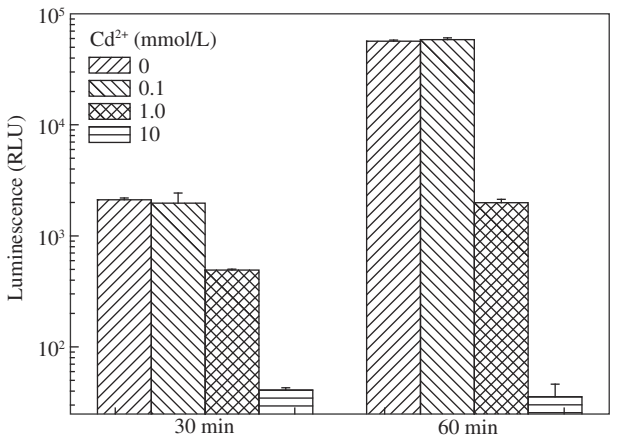

Fig. 4 – Light emission of pTHE30–*E. coli* after exposed to 0, 0.1, 1, or 10 mmol/L Cd^{2+} for 30 and 60 min with 100 nmol/L additional Hg^{2+}.

for wells with Cd^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+}, and Al^{3+} concentration from 10^{-5} to 1 mmol/L, significant luminescence was detected after the addition of 100 nmol/L Hg^{2+}, and the luminescence value of each well was similar to that of the well with just 100 nmol/L Hg^{2+}, except for the well with 1 mmol/L Cd^{2+}. The results of 0.1, 1, and 10 mmol/L Cd^{2+} are shown in Fig. 4; for higher heavy metal concentrations of Cd^{2+}, the cell activity may be affected and result in a false negative effect.

The luminescence of wells with 0.1 mmol/L or 1 mmol/L Cd^{2+} increased after the addition of 100 nmol/L Hg^{2+}, but the luminescence of wells with 1 mmol/L Cd^{2+} was much lower, and the well containing 10 mmol/L Cd^{2+} gave luminescence close to the background level. In a typical experiment, pTHE30–*E. coli* could grow in medium with 0.01 mmol/L Cd^{2+}, but the OD600 value did not increase in mediums with 0.1 mmol/L, 1 mmol/L, and 10 mmol/L Cd^{2+}. Therefore, the recombinant strain was able to function in 0.1 mmol/L Cd^{2+} but not with constant exposure, while the activity of this strain was suppressed when Cd^{2+} was above 1 mmol/L. For higher heavy metal concentrations, in this case 10 mmol/L Cd^{2+}, cells might show loss of activity and show no response to Hg^{2+}, and result in a false negative problem.

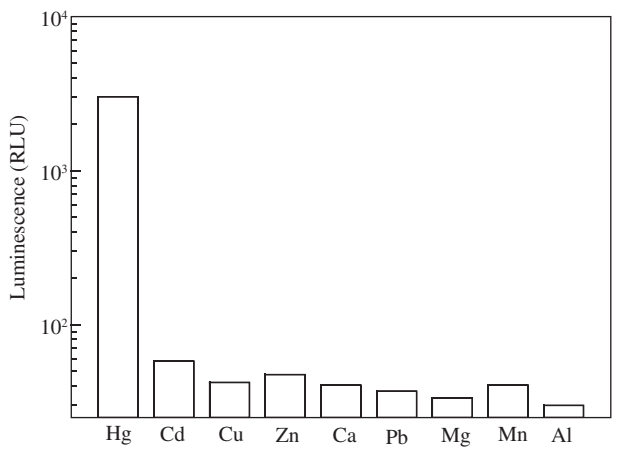

Fig. 3 – Specificity of pTHE30–*E. coli* in the presence of different heavy metals. Data shown were the maximum luminescence of heavy metal in a range of 10^{-5}–1 mmol/L.

2.4. Detection mode by internal standard method

In a luminescence test, 4 luminescence values were needed, including RLU_N (luminescence of negative control, without water sample and analyte), RLU_P (luminescence of positive control, with analyte only), RLU_S (luminescence of water sample), and RLU_I (luminescence of water sample with additional analyte). An extra benefit of applying the internal standard method in the recombinant luminescent bacteria method is that it will be helpful in determining the concentration range of the target analyte in the test sample.

To illustrate how this method works, the data of 60 min exposure time in Fig. 2 were used, since the data of 60 min showed more significant difference than those at 30 min. The dose–response curve of this recombinant strain was quasi-trapezoidal in shape, and the curve entered into a steady region from 100 to 1000 nmol/L Hg^{2+}. Therefore, the internal standard sample should be an aqueous mixture with Hg^{2+} within this concentration range. By comparing the values of RLU_N, RLU_P, RLU_S, and RLU_I, the analyte concentration (C_a) range in the test sample can be determined, as shown in Table 1. In the case of

Table 1 – Interpretation of luminescence test results applying internal standard method.

Results	Hg concentration (nmol/L)
$RLU_N = RLU_S < RLU_I \leq RLU_P$	$C_a < 5$ [a]
$RLU_N < RLU_S < RLU_I \leq RLU_P$	$5 < C_a < 100$
$RLU_N < RLU_S = RLU_I \leq RLU_P$	$100 < C_a < 1000$
$RLU_N < RLU_S = RLU_I < RLU_P$	$1000 < C_a < 10000$
$RLU_N = RLU_S = RLU_I < RLU_P$	$C_a > 10000$ or high cytotoxicity

RLU_N: luminescence of negative control without water sample and analyte; RLU_P: luminescence of positive control, with analyte only ater sample, water sample with additional analyte, positive control; RLU_S: luminescence of water sample; RLU_I: luminescence of water sample with additional analyte.

[a] Values were derived from dose response curve.

Table 2 – Recombinant luminescence bacteria test by internal standard method.

	Sample	$HgCl_2$ (nmol/L)	Luminescence	$C_{Hg^{2+}}$ (nmol/L)
RLU_N	Water	0	48.63	
RLU_{S0} [a]	Effluent	2.0	30.82	$C_a < 5$
RLU_{I0} [b]		2.0 + 100	61133	
RLU_{S1} [c]		2.0 + 20	7355.2	$5 < C_a < 100$
RLU_{I1}		2.0 + 20 + 100	56023	
RLU_{S2}		2.0 + 1000	60072	$100 < C_a < 1000$
RLU_{I2}		2.0 + 1000 + 100	59518	
RLU_{S3}		2.0 + 2000	202.19	$1000 < C_a < 10000$
RLU_{I3}		2.0 + 2000 + 100	262.89	
RLU_{S4}		2.0 + 100 000	59.81	$C_a > 10000$ or high toxicity
RLU_{I4}		2.0 + 100 000 + 100	34.13	
RLU_{S5} [a]		2.0 + 1000	2393.1	$100 < C_a < 1000$
RLU_{I5} [d]		2.0 + 1000 + 100	2506.4	
RLU_P	Water	1 μM	66045	

[a] RLU_{S0} was the luminescence of sample 0 contained 2.0 nmol/L Hg (background concentration).
[b] Sample I0 was internal standard sample 0, obtained by adding 100 nmol/L Hg to sample 0.
[c] Sample 1 was obtained by adding 20 nmol/L Hg to sample 0.
[d] Sample 5 contained 1 mmol/L Cd^{2+}.

environmental monitoring, the amount of analyte in the internal standard sample can be derived from the limit value.

2.5. *Water sample testing*

A water sample was collected from the discharge point of the Qinghe Sewage Treatment Plant after the renewable water treatment unit, and the background mercury concentration was determined to be 2.0 nmol/L by the atomic fluorescent spectrometry method. The original sample and samples with different concentrations of mercury added were tested without any pretreatment; sample 0 was the original water sample, 1, 2, 3, 4 had mercury added to final concentrations of 50 nmol/L, 1 μmol/L, 20 μmol/L, and 100 μmol/L; sample 5 contained 100 nmol/L Hg^{2+} and 1 mmol/L Cd^{2+}. The cell suspension was prepared in accordance with Section 2.2. Luminescence data shown in Table 2 were measured after 60 min of exposure.

3. Discussion and conclusions

Efficient fusion of a heavy metal operon and reporter gene is the key point for construction of a heavy metal sensing bioreporter. In this study, a mercury sensing gene operon, containing the mercury-resistant gene *mer*R and the promoter/operator region of the *mer* gene operon, was isolated from marine *P. putida* SP1 and inserted into the upper stream of the prompterless *lux* gene in the pUCD615 plasmid. A bacterium strain carrying this recombinant plasmid showed high specificity and sensitivity to Hg^{2+}, while other metals, such as Cd^{2+}, Cu^{2+}, Zn^{2+}, Ca^{2+}, Pb^{2+}, Mg^{2+}, Mn^{2+} and Al^{3+}, did not interfere with the luminescence assay. The detection range of Hg^{2+} was from 5 nmol/L to 10 μmol/L, and distinct luminescence could be detected in 30 min.

The use of recombinant luminescent bacteria based on the *mer* gene had the potential to be a proper method for online monitoring of bioavailable mercury, but many issues needed more research before field application (Charrier et al., 2011). One of the most important issues was to avoid the false negative problem. For this purpose, the internal standard method was selected.

When the internal standard method was applied in luminescence testing, luminescence caused by addition of the target substance (100 nmol/L Hg^{2+}) in a low cytotoxicity sample (0.1 mmol/L Cd^{2+}) was the same as one with only the pure substance (containing Hg^{2+} only), while similar but lower luminescence was observed in a medium cytotoxicity sample (1 mmol/L Cd^{2+}), and only limited response in a high cytotoxicity sample (10 mmol/L Cd^{2+}). In a practical water sample test, a parallel test with additional target substance will be appropriate for estimating the cytotoxicity of the water sample and enables the avoidance of false negative interference. In this experiment, the parallel test sample was an internal sample with 100 nmol/L Hg^{2+}, which was helpful in discovering false negative samples (sample 4 in Section 2.5). This method can be used in samples with low or medium cytotoxicity (sample 5 in Section 2.5). As for high cytotoxicity samples, direct use of the recombinant luminescent bacteria method is not applicable, and some adjustments will be needed.

Recombinant luminescence bacteria could assess the bioavailability of heavy metals in the environment; light emission is dependent on the active amount of the target compound in the microbial cells and the energy supply. The recombinant luminescent strain in this research could be used to sensitively and specifically measure the bioavailability of Hg^{2+} in water samples. The influence of interfering substances such as cadmium was limited, and the false positive problem was under control. To avoid the false negative problem, using the internal standard method in a parallel test with additional mercury is not only easy but also helpful in identifying false negative samples. Environmental samples may contain a variety of heavy metals, so that a combination of different recombinant luminescent bacteria strains will be needed for practical monitoring of heavy metal exposure risk.

Acknowledgments

We thank Professor Lingxin Chen (Chinese Academy of Science) for supplying *P. putida* strain SP1. This work was supported by the National Natural Science Foundation of China (No. 21377065), and the 863 National High-Tech Research and Development Program (No. 2014AA06A506).

REFERENCES

Barkay, T., Gillman, M., Turner, R.R., 1997. Effects of dissolved organic carbon and salinity on bioavailability of mercury. Appl. Environ. Microbiol. 63 (11), 4267–4271.

Barkay, T., Miller, S.M., Summers, A.O., 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27 (2–3), 355–384.

Baumstark-Khan, C., Cioara, K., Rettberg, P., Horneck, G., 2005. Determination of geno- and cytotoxicity of groundwater and sediments using the recombinant SWITCH test. J. Environ. Sci. Health A 40 (2), 245–263.

Bogdanova, E., Minakhin, L., Bass, I., Volodin, A., Hobman, J.L., Nikiforov, V., 2001. Class II broad-spectrum mercury resistance transposons in Gram-positive bacteria from natural environments. Res. Microbiol. 152 (5), 503–514.

Charrier, T., Durand, M.J., Jouanneau, S., Dion, M., Pernetti, M., Poncelet, D., et al., 2011. A multi-channel bioluminescent bacterial biosensor for the on-line detection of metals and toxicity. Part I, design and optimization of bioluminescent bacterial strains. Anal. Bioanal. Chem. 400 (4), 1051–1060.

Close, D.M., Patterson, S.S., Ripp, S., Baek, S.J., Sanseverino, J., Sayler, G.S., 2010. Autonomous bioluminescent expression of the bacterial luciferase gene cassette (lux) in a mammalian cell line. PLoS One 5. http://dx.doi.org/10.1371/journal.pone.0012441.

Close, D., Xu, T.T., Smartt, A., Rogers, A., Crossley, R., Price, S., et al., 2012. The evolution of the bacterial luciferase gene cassette (lux) as a real-time bioreporter. Sensors 12 (1), 732–752.

De, J., Ramaiah, N., Vardanyan, L., 2008. Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Mar. Biotechnol. 10 (4), 471–477.

Du, M., Wei, D.M., Tan, Z.W., Lin, A.W., Du, Y.G., 2015. Predicted no-effect concentrations for mercury species and ecological risk assessment for mercury pollution in aquatic environment. J. Environ. Sci. 27 (2), 74–80.

Endo, G., Yamagata, T., Narita, M., Huang, C.C., 2003. Bioluminescence biosensor for the detection of organomercury contamination. Acta Biotechnol. 23 (2–3), 123–129.

Escher, B., Leusch, F., 2012. Bioanalytical Tools in Water Quality Assessment. IWA Publishing, New York.

Gupta, R.K., Patterson, S.S., Ripp, S., Simpson, M.L., Sayler, G.S., 2003. Expression of the *Photorhabdus luminescens lux genes* (*luxA*, *B*, *C*, *D*, and *E*) in *Saccharomyces cerevisiae*. FEMS Yeast Res. 4 (3), 305–313.

Hakkila, K., Maksimow, M., Karp, M., Virta, M., 2002. Reporter genes lucFF, luxCDABE, gfp, and dsred have different characteristics in whole-cell bacterial sensors. Anal. Biochem. 301 (2), 235–242.

Hakkila, K., Green, T., Leskinen, P., Ivask, A., Marks, R., Virta, M., 2004. Detection of bioavailable heavy metals in EILATox-Oregon samples using whole-cell luminescent bacterial sensors in suspension or immobilized onto fibre-optic tips. J. Appl. Toxicol. 24 (5), 333–342.

Hakkila, K.M., Nikander, P.A., Junttila, S.M., Lamminmaki, U.J., Virta, M.P., 2011. Cd-Specific mutants of mercury-sensing regulatory protein merr, generated by directed evolution. Appl. Environ. Microbiol. 77 (17), 6215–6224.

Harkins, M., Porter, A.J.R., Paton, G.I., 2004. The role of host organism, transcriptional switches and reporter mechanisms in the performance of Hg-induced biosensors. J. Appl. Microbiol. 97 (6), 1192–1200.

Huang, X.X., He, M., Luo, H., Shi, H.C., Cai, Q., 2009. Construction of the pUCD-recA recombinate luminescence bacterium to evaluate the genetoxicty of environment pollutants. Chin. J. Environ. Sci. 30 (6), 1722–1726.

Hynninen, A., Virta, M., 2010. Whole-cell bioreporters for the detection of bioavailable metals. Adv. Biochem. Eng. Biotechnol. 118, 31–63.

Hynninen, A., Tõnismann, K., Virta, M., 2010. Improving the sensitivity of bacterial bioreporters for heavy metals. Bioeng. Bugs 1 (2), 132–138.

Iohara, K., Iiyama, R., Nakamura, K., Silver, S., Sakai, M., Takeshita, M., et al., 2001. The mer operon of a mercury-resistant *Pseudoalteromonas haloplanktis* strain isolated from Minamata Bay, Japan. Appl. Microbiol. Biotechnol. 56 (5–6), 736–741.

Ivask, A., Virta, M., Kahru, A., 2002. Construction and use of specific luminescent recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium, zinc, mercury and chromium in the soil. Soil Biol. Biochem. 34 (10), 1439–1447.

Kholodii, G.Y., Yurieva, O.V., Gorlenko, Z.M., Mindlin, S.Z., Bass, I.A., Lomovskaya, O.L., et al., 1997. Tn5041, a chimeric mercury resistance transposon closely related to the toluene degradative transposon Tn4651. Microbiology 143 (8), 2549–2556.

Mathema, V.B., Thakuri, B.C., Sillanpää, M., 2011. Bacterial *mer* operon-mediated detoxification of mercurial compounds, a short review. Arch. Microbiol. 193 (12), 837–844.

Moore, B., 1960. A new screen test and selective medium for the rapid detection of epidemic strains of *Staph aureus*. Lancet 276 (7148), 453–458.

Nascimento, A.M.A., Chartone-Souza, E., 2003. Operon mer, bacterial resistance to mercury and potential for bioremediation of contaminated environments. Genet. Mol. Res. 2 (1), 92–101.

Rasmussen, L.D., Sørensen, S.J., Turner, R.R., Barkay, T., 2000. Application of a *mer-lux* biosensor for estimating bioavailable mercury in soil. Soil Biol. Biochem. 32 (5), 639–646.

Roda, A., Pasini, P., Mirasoli, N., Guardigli, M., Russo, C., Musiani, M., et al., 2001. Sensitive determination of urinary mercury(II) by a bioluminescent transgenic bacteria-based biosensor. Anal. Lett. 34 (1), 29–41.

Rogowsky, P.M., Close, T.J., Chimera, J.A., Shaw, J.J., Kado, C.I., 1987. Regulation of the vir genes of agrobacterium-tumefaciens plasmid Ptic58. J. Bacteriol. 169 (11), 5101–5112.

Selifonova, O., Burlage, R., Barkay, T., 1993. Bioluminescent sensors for detection of bioavailable Hg(Ii) in the environment. Appl. Environ. Microbiol. 59 (9), 3083–3090.

Song, Y.Z., Li, G.H., Thornton, S.F., Thompson, I.P., Banwart, S.A., Lerner, D.N., et al., 2009. Optimization of bacterial whole cell bioreporters for toxicity assay of environmental samples. Environ. Sci. Technol. 43 (20), 7931–7938.

Sørensen, S.J., Burmølle, M., Hansen, L.H., 2006. Making bio-sense of toxicity, new developments in whole-cell biosensors. Curr. Opin. Biotechnol. 17 (1), 11–16.

Thouand, G., Horry, H., Durand, M.J., Picart, P., Bendriaa, L., Daniel, P., et al., 2003. Development of a biosensor for on-line detection of tributyltin with a recombinant bioluminescent *Escherichia coli* strain. Appl. Microbiol. Biotechnol. 62 (2–3), 218–225.

Trang, P.T.K., Berg, M., Viet, P.H., Van Mui, N., Van Der Meer, J.R., 2005. Bacterial bioassay for rapid and accurate analysis of arsenic in highly variable groundwater samples. Environ. Sci. Technol. 39 (19), 7625–7630.

Van der Meer, J.R., Tropel, D., Jaspers, M., 2004. Illuminating the detection chain of bacterial bioreporters. Environ. Microbiol. 6 (10), 1005–1020.

Yagur-Kroll, S., Belkin, S., 2011. Upgrading bioluminescent bacterial bioreporter performance by splitting the lux operon. Anal. Bioanal. Chem. 400 (4), 1071–1082.

Zhang, X.Y., He, M., Huang, X.X., Shi, H.C., 2007. Construction of recombinant luminescent bacteria for genetic toxicity evaluation of environmental pollutants. Proceedings of International Workshop on Monitoring and Sensor for Water Pollution Control. Beijing, China. June 13–14.

Zhang, W.W., Chen, L.X., Liu, D.Y., 2012. Characterization of a marine-isolated mercury-resistant *Pseudomonas putida* strain SP_1 and its potential application in marine mercury reduction. Appl. Microbiol. Biotechnol. 93 (3), 1305–1314.