Journal of Bioscience and Bioengineering
VOL. xxx No. xxx, xxx, xxxx

www.elsevier.com/locate/jbiosc

Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells

Dan Wang,[1,*] Yanan Zheng,[1] Xiaosu Fan,[2] Lina Xu,[3] Ting Pang,[1] Ting Liu,[1] Legui Liang,[1] Shan Huang,[1] and Qi Xiao[1]

College of Chemistry and Materials, Guangxi Key Laboratory of Natural Polymer Chemistry and Physics, Nanning Normal University, Nanning 530001, PR China,[1] Experimental Center of College of Agriculture, Guangxi University, Nanning 530005, PR China,[2] and Institute of Chemical Industry of Forestry Products, CAF, Nanjing 210042, PR China[3]

Received 30 June 2019; accepted 10 August 2019
Available online xxx

Mercury pollution has always been a huge threat to human health due to its significant toxicity. Thus, it's the continuing goal to obtain new mercury detection techniques that are cost-effective, operational stable, performance efficient, and applicable to the environmental and biological milieus. In this research, the soluble pigment pyocyanin with anti-bacterial and anti-fungal activities, the biosynthesis pathway of which was engineered under the regulation of Hg^{2+}-dependent transcriptional activator MerR, was firstly used as the visual detection signal in the whole-cell biosensor. The engineered biosensor displayed optical sensing window and a good linearity for Hg^{2+} in the range of 25–1000 nM, and the detection limit could reach as low as 10 nM. It permitted on-site detection of bioavailable Hg^{2+} with extraordinary selectivity and could resist the interferences of extra metal ions. What's more, the developed biosensor performed function well in a wide pH range (pH 4–10) as well as the environmental water. By fully imitating and utilizing the biosystems from nature, the engineered colorimetric biosensor has great economic and performance advantages over most chemosensors as well as whole-cell biosensors in the practical application of detecting Hg^{2+} in the contaminated aquatic systems.

[**Key words:** Mercury detection; Colorimetric biosensor; Transcription factor MerR; Biosynthesis of pyocyanin; Visual detection signal]

Mercury has been defined by the World Health Organization (WHO) as one of the top ten globally dangerous chemicals to human health when present in drinking water (1). Even trace mercury exposure will pose severe toxic effects on the organ systems in particular to the nervous system (2–4). Therefore, it's constantly pursued to develop efficient detection methods for mercury to protect the public health from the strong biological toxicity. Traditional quantitative techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS), atomic absorption spectrometry (AAS), and atomic fluorescence spectrometry (AFS) can analyze the Hg^{2+} content in solution sensitively with high accuracy (5). However, these instrumental analyses inevitably rely on expensive equipment, complicated pretreatment, and quality workplace that make them impractical to be used as portable devices for on-site quantification. These problems are partially solved by the large amount of chemosensors developed based on nanoparticle, polymer, organic, and biological molecules in recent years (6–11). Most of the chemosensors had good performances on sensitivity and selectivity, whereas the intensive integration and poor reproducibility generally render them difficult for large-scale determination. In this context, the potentially economic, convenient, and repeatable techniques are urgently needed for the detection of mercury contamination.

The whole-cell biosensor continued to spring up as the alternative strategies for the detection of heavy metal ions over the last few decades (12). By genetic engineering of microorganisms such as algas, fungi, and bacteria, the whole-cell biosensors integrating the signal sensing and reporter apparatuses within a living cell are cost-effective, operational stable, and performance efficient. Most importantly, the whole-cell biosensors can be largely prepared in a short-term by simple and inexpensive cultivation process, which are particularly suitable for the high-throughput in situ determination. Thus, more and more whole-cell biosensors have been developed in recent years (13–18). Most of them have high sensitivity towards target metal ions by using the bioluminescence and fluorescence as the reporter apparatuses. Whereas, it should be noted that these reporter apparatuses in the biosensors have some drawbacks (19). The measuring bioluminescence produced by bacterial luciferase is heat-labile, and firefly luciferase requires external substrates. The fluorescent reporter proteins require a longer time to emit a stable fluorescence, and they always have the needs to distinguish auto-fluorescence or background fluorescence from the real fluorescent signal in cells during measurement. Thus, the whole-cell biosensors with reporter apparatuses stable and convenience of measurement are still needed to meet the requirements of environmental samples monitoring.

In this research, the colorimetric biosensor for Hg^{2+} was engineered by employing the soluble pigment pyocyanin as the visual and measurable output signal. The genes *phzM* and *phzS*, which are the important factors in the biosynthesis of pyocyanin, are under

* Corresponding author. Tel.:/Fax: +86 771 3908065.
E-mail address: wangdan1989323@126.com (D. Wang).

1389-1723/$ – see front matter
https://doi.org/10.1016/j.jbiosc.2019.08.005

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005

the regulation of the Hg^{2+}-dependent transcriptional regulator MerR in the cell of *Pseudomonas aeruginosa* PAO1 (Fig. 1) (20–22). As a result, the genetic signal network was successfully synthesized by using the transcription factor MerR as the signal sensing apparatus and the synthesis pathway of pyocyanin as the signal output apparatus. The signal molecule pyocyanin is soluble pigment that exhibits blue-green color and measurable absorbance at 520 nm after solvent treatment. As a result, the Hg^{2+} contamination can be preliminary estimated by the blue-green color in the medium, which also can be further quantified by determining the absorbance at 520 nm. In addition, the pyocyanin has good anti-bacterial and anti-fungal activities, which could protect the detection process against the interference of abundant microorganisms in the real water samples. Therefore, the present biosensor is practically useful in detecting Hg^{2+} pollution with a visual, stable, economic, and effective manner.

MATERIALS AND METHODS

Strains, plasmids, media, and reagents *Escherichia coli* DH5α strain was used as the host cell for recombinant DNA manipulation. *P. aeruginosa* PAO1 strain was used as the host cell for the engineered biosensor. Plasmid pAK1900 carrying ampicillin resistance was used to express fusion gene *merR*-P*mer*-*phzM*-P*mer*-*phzS*. *E. coli* DH5α and *P. aeruginosa* PAO1 cells were grown in Luria Bertani (LB) medium [1% (w/v) tryptone, 0.5% (w/v) yeast extract, and 1% (w/v) sodium chloride] containing 50 μg/ml of ampicillin. The *P. aeruginosa* PAO1 cells were also cultivated in the King A medium [2% (w/v) tryptone, 0.14% (w/v) magnesium chloride, and 1% (w/v) potassium sulfate] to maximize pyocyanin production. The analytical standard solutions of Hg^{2+} and other metal ions (Cd^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, and Cr^{3+}) were purchased from Shanghai Aladdin Biochemical Technology Co. Ltd., Shanghai, China. The metal ions Hg^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+}, and Cu^{2+} are existed in the form of nitrates in 1 mol/L or 1% nitric acid. The metal ion Cr^{3+} is existed in the form of chloride in 1 mol/L hydrochloric acid. The authentic standard of pyocyanin was purchased from ApexBio Technology. The high purity deionized water (resistivity 18 MΩ cm), produced by the Millipore water purification system, was used throughout the experiments.

Construction of the colorimetric mercury sensing biosensor The plasmid of biosensor was constructed by using pAK1900 as expression vector in the following steps (Fig. S1). Firstly, the DNA fragment including *merR* gene and its bidirectional promoter *mer* (*merR*-P*mer*, GenBank: Z00027.1) was synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China), and it was amplified by PCR reaction with primer I and primer II. The DNA fragment of PhzM protein (GenBank: PTC38886.1) was amplified with primer III and primer IV. Then, the aforementioned two fragments were connected by overlap PCR using primer I and primer IV. The overlap PCR product was cloned into the HindIII and BamHI sites of pAK1900 vector, which yielded the *merR*-P*mer*-*phzM*-pAK1900 construct. Secondly, the *mer* promoter was amplified with primer V and primer VI, and the gene encoding PhzS protein (GenBank: PTC38903.1) was amplified with primer VII and primer VIII. The two fragments were connected by overlap PCR with primer VI and primer VIII, and they were cloned into the BamHI site of *merR*-P*mer*-*phzM*-pAK1900 construct. The final construct *merR*-P*mer*-*phzM*-P*mer*-*phzS*-pAK1900 was confirmed by sequencing and transformed into the competent cells of *P. aeruginosa* PAO1 by chemical method. Primers used in the construction of expression vector are listed in Table S1.

Cultivation of the colorimetric mercury sensing biosensor *P. aeruginosa* PAO1 cells containing the final construct *merR*-P*mer*-*phzM*-P*mer*-*phzS*-pAK1900 were grown in LB medium with 50 μg/ml of ampicillin. They were cultivated overnight at 37°C with constantly shaking at 250 rpm. The overnight bacteria cells were further cultivated in the King A medium after inoculation at a proportion of 1:100. They were continued to cultivate for 1 h before adding Hg^{2+} or other metal ions. When adding suitable concentration of Hg^{2+} into the medium, certain amount of pyocyanin will be produced by the cells of biosensor after cultivation. The growth rates of the cells in the presence of different concentration of Hg^{2+} were also monitored in the following 12 h by determining their optical density of OD_{600}.

Liquid chromatography-mass spectrometry analysis of signal molecule pyocyanin of colorimetric biosensor The pyocyanin produced by the engineered colorimetric biosensor was characterized by liquid chromatography-mass spectrometry (LC-MS) analysis in the following procedures. Firstly, the supernatant of the cell samples was obtained by centrifugation, and 5 ml supernatant was mixed with 3 ml chloroform. The pyocyanin from organic phase was pooled and evaporated to dry by rotary evaporation. The dried pyocyanin was resuspended in methanol, and the insoluble material was removed by high-speed centrifugation and 0.22 μm membrane. Finally, the dissolved pyocyanin was subjected to LC-MS analysis on Waters UPLC ICLASS-XEVOG2-XS QTOF equipment (Waters, Milford, MA, USA). The pyocyanin was run on the ultra-performance liquid chromatography (UPLC) system equipped with C18 analytical column (i.d., 1.7 μm particle size, 2.1 × 50 mm) with mobile phases A (99.9% water with 0.1% formic acid) and B (100% acetonitrile). Pyocyanin was separated with a gradient of 4 min of 95% B followed by 2 min of 10% B at a constant flow rate of 0.4 ml/min. The mass spectra were recorded in the positive ion spray ionization (ESI^+) mode with the following conditions: nebulizing gas, 50 L/h; source temperature, 100°C; and capillary voltage, 3.00 KV.

Mercury detection with the colorimetric biosensor For the evaluation of detection sensitivity towards Hg^{2+}, graded concentrations of Hg^{2+} (0, 10, 25, 50, 100, 250, 500, 1000, and 5000 nM) were added into the King A cultural medium after the overnight culture inoculation at a proportion of 1:100 for 1 h. After induced by Hg^{2+} for 12 h, the cell samples were taken for analysis. For the evaluation of detection selectivity towards Hg^{2+}, some other metal ions, such as Cd^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, and Cr^{3+} at the concentration of 500 nM, and the mixture of 100 nM Hg^{2+} with each of them at the concentration of 100 nM were added to replace Hg^{2+} into the King A cultural medium, respectively. The cell samples were continued to cultivate for 12 h before taking for analysis, too. To evaluate the effect of pH values on the detection of Hg^{2+}, the overnight culture was continued to cultivate following a 1:100 inoculation in the King A medium with different pH values (pH= 4, 5, 6, 7, 8, 9, and 10). The cells of biosensor were initiated after cultivation for 1 h by adding 100 nM Hg^{2+} into the cultural medium, and they were continued to cultivate for another 12 h before taking for analysis. In the analysis of environmental water, the lake water samples were used to replace the high purity deionized water for the preparation of mercury solutions. The remaining steps were performed as described in the mercury sensitivity assay.

All the cell samples were prepared by quintuplicate, and the pyocyanin in them was quantified according the previous research (23). Briefly, the supernatant of the cell samples was obtained by centrifugation, and per 5 ml supernatant was mixed

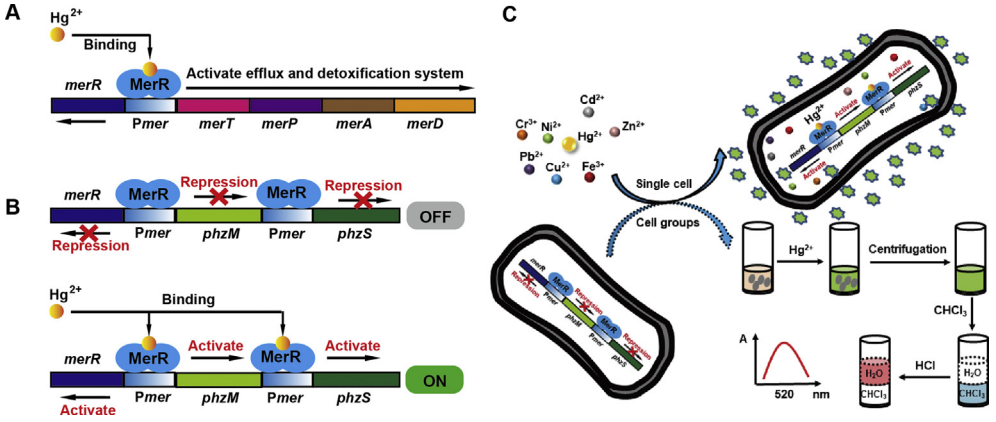

FIG. 1. The design of colorimetric mercury sensing biosensor. (A) The archetype of genetic organization of the *mer* operon locus on transposable elements *Tn21* and *Tn501*. In the presence of Hg^{2+}, the MerR dimer undergoes a conformational change that enables RNA polymerase to initiate the transcription of the downstream efflux and detoxification genes. (B) The genetic organization of the signal sensing and output apparatuses of the biosensor. In the absence of Hg^{2+}, the expressions were repressed for the genes *phzM* and *phzS*. Once Hg^{2+} recognized and bound by MerR dimer, the expressions were activated for the genes *phzM* and *phzS*. (C) Visual and quantitative detection of Hg^{2+} by the engineered biosensor with the transcription factor MerR regulated biosynthesis of pyocyanin.

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005

with 3 ml chloroform. Then, the pyocyanin from organic phase was extracted with 1 ml 0.2 M HCl, which would give a pink to deep red color due to the amount of pyocyanin. The quantification of pyocyanin was performed by measuring its absorbance at 520 nm in the acidic solution on the UV/VIS spectrophotometer (Shimadzu RF3601, Shimadzu, Kyoto, Japan). The response signal of the biosensor towards different concentration of Hg^{2+} was calculated by the following equation:

$$\text{Response signal} = \frac{(OD_{520\ nm,\ 12\ h} - OD_{520\ nm,0})_{pyocyanin}}{(OD_{600\ nm,\ 12\ h} - OD_{600\ nm,0})_{cells}} \quad (1)$$

RESULTS AND DISCUSSION

The design of the colorimetric mercury sensing biosensor In the metal ions seriously polluted environment, some prokaryotes can survive well by utilizing internal metal-sensing transcription factors to quickly sense the stimulation and efficiently regulate the expression of the metal-resistance genes. The Hg^{2+}-dependent transcriptional activator MerR, a mercuric ion resistance regulator, which exhibits extraordinary sensitivity towards Hg^{2+} with high selectivity and can tightly control the detoxification system *merTP(C)AD* through the bidirectional *mer* promoter (24) (Fig. 1A). Inspired by the regulation mechanism of metalloprotein MerR, the Hg^{2+}-specific biosensor was designed through replacing the structural genes *merTP(C)AD* by the genes of *phzM* and *phzS* (Fig. 1B). The phenazine-specific methyltransferase PhzM and flavin-containing monooxygenase PhzS are two phenazine-modifying enzymes that can greatly facilitate the biosynthesis of pyocyanin in *P. aeruginosa* (21). The pyocyanin is the phenazine-derived soluble pigment that exhibits blue-green color and measurable absorbance at 520 nm after solvent treatment, which can provide the visual and characteristic signal in the detection of Hg^{2+} (25) (Fig. 1C).

The construction of the colorimetric mercury sensing biosensor Based on the design, the plasmid of the developed biosensor was successfully constructed following the procedures shown in Fig. S1. As shown in Fig. S2, the genes *merR*-P*mer* (506 bp), *phzM* (1008 bp), P*mer* (71 bp), and *phzS* (1209 bp) were separately amplified by PCR reactions. Then, the fragments *merR*-P*mer*-*phzM* (1514 bp) and P*mer*-*phzS* (1280 bp) were obtained by overlap PCR, and they were ligated into the pAK1900 vector by the HindIII and BamHI sites. After confirmed by sequencing, the mercury sensing biosensor was successfully engineered with the final construct *merR*-P*mer*-*phzM*-P*mer*-*phzS*-pAK1900 transformed into the competent cells of *P. aeruginosa* PAO1. In the absence of Hg^{2+}, the transcriptional regulator MerR bound to the *mer* promoter was in the inactivate state, which will repress the transcription of the downstream genes *phzM* and *phzS*. Upon Hg^{2+} binding, the metalloprotein MerR undergoes a conformational change that will activate the transcription of the downstream genes *phzM* and *phzS*. With the phenazine-modifying enzymes PhzM and PhzS largely expressed, more pyocyanin will be synthesized by the cells of biosensor. As a result, the Hg^{2+} in the medium can be preliminary estimated by the blue-green color displayed by the pyocyanin, which also can be further quantified by determining the absorbance at 520 nm after solvent treatment.

LC-MS analysis of signal molecule pyocyanin of colorimetric biosensor Under the stimulation of Hg^{2+}, the cells of biosensor revealed elevated production of blue-green pigment in the cultural medium. To identify the color changes were indeed caused by the pyocyanin, the pigment in the cultural medium was extracted and analyzed by LC-MS, and the results were compared to those of the authentic standard of pyocyanin (Fig. S3). In the UPLC analysis, the pigment in the supernatant kept consistent retention time with the authentic standard (retention time 0.88 min). The full-scan positive ion spectra of the pigment in the

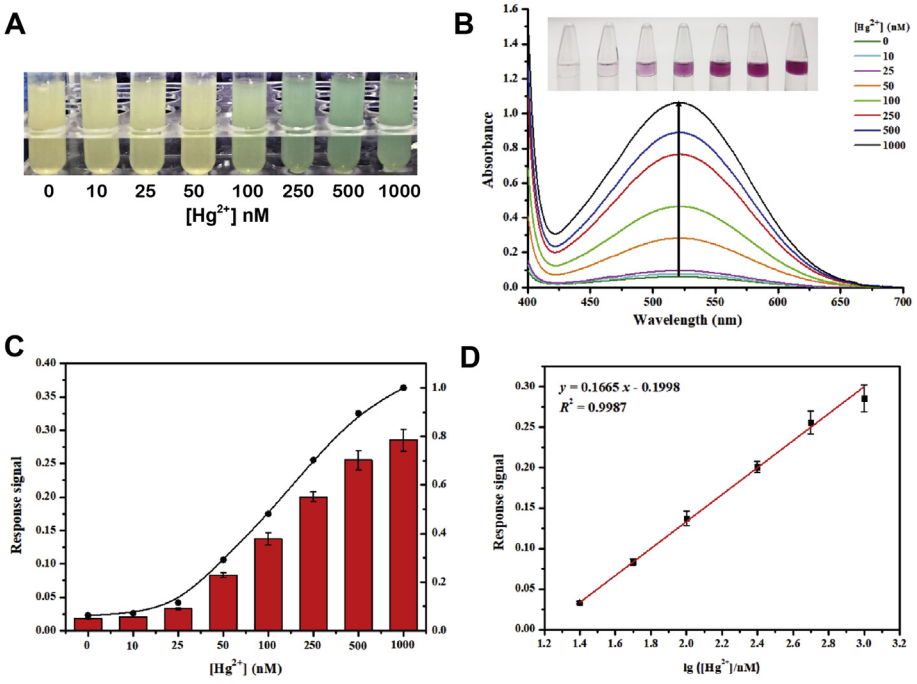

FIG. 2. The response of biosensor towards different concentrations of Hg^{2+}. (A) The increased production of pyocyanin generated by the cells of biosensor induced with graded concentrations of Hg^{2+} in the range of 0–1000 nM. (B) The produced pyocyanin exhibited pink to deep red color and exhibited increased absorbance at 520 nm after extraction and treatment. (C) The increased response signal produced by the cells of biosensor induced with graded concentrations of Hg^{2+} in the range of 0–1000 nM. (D) The relationship between the response signal and concentration of Hg^{2+} from 25 to 1000 nM with the linear regression equation $y = 0.1665x\text{-}0.1998$ ($R^2 = 0.9987$), where x and y represent the log concentration of Hg^{2+} and the response signal. All the data are the means of five independent experiments. Error bars correspond to the standard deviations.

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005

supernatant indicated that the ions were $[M + H]^+$ ions at *m*/*z* 211.09, which match the theoretical molecular mass of pyocyanin (calculated MW= 210.23 g/mol). All the results highlighted that the engineered biosensor was successfully in sensing Hg^{2+} by manipulation the biosynthesis pathway of pyocyanin in *P. aeruginosa* PAO1.

Mercury sensitivity detection with the colorimetric biosensor The engineered biosensor could grow well (Fig. S4) and provide a visual sensing window for Hg^{2+} in the range of 10-1000 nM (Fig. 2A and B). As shown in Fig. 2B and C, in the presence of 10 nM Hg^{2+}, the cells of the biosensor displayed detectable and higher response signal than that in the absence of Hg^{2+}, which was much more sensitivity than the most previously reported chemosensors as well as the whole-cell biosensors (7,8,26–29). The detection limit was also in the range of safety levels in drinking water (5–30 nM) for Hg^{2+} recommended by WHO and other governmental protection agencies worldwide (29). It indicated that the engineered biosensor could basically meet the requirements of environment samples monitoring. With the concentration of Hg^{2+} continued increasing, the cells of biosensor showed enhanced production of pyocyanin in the cultural medium (Fig. 2A). After simple treatment, the produced pyocyanin in the cell samples gave the pink to deep red color and exhibited increased absorbance at 520 nm (Fig. 2B). Regression analysis demonstrated a good linear relationship between the absorbance and the log concentration of Hg^{2+} from 25 to 1000 nM with the correlation coefficient of $R^2 = 0.9987$ (Fig. 2D). When the concentration of Hg^{2+} exceeded 5000 nM, the signal of the biosensor was greatly affected and decreased to an extremely low level (data not shown). It is speculated that the impairment of signal was attributed to the huge toxicity of Hg^{2+} that exceeded the tolerance ability of the engineered biosensor.

Mercury selectivity detection with the colorimetric biosensor The metalloprotein MerR is the Hg^{2+}-specific transcriptional regulator, which has an extremely high affinity for Hg^{2+} and can distinguish Hg^{2+} easily from other metal ions. Through employing the sensing module of the transcription factor MerR, the engineered whole-cell biosensor showed extraordinary selectivity to Hg^{2+} in the aquatic systems. As shown in Fig. 3A, the biosensor responded silently to most metal ions such as Cd^{2+}, Pb^{2+}, Zn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, and Cr^{3+}. In contrast, it displayed intense signal in the presence of the same concentration of Hg^{2+}. What's more, the signal of the biosensor towards Hg^{2+} was not influenced by other metal ions. In the presence of interference metal ions, the biosensor still showed intense signals which could be comparable to the signal determined in the presence of Hg^{2+} alone (Fig. 3B). As is well-known, the mercury pollution is always present with other heavy metal ions. Therefore, the good selectivity and antijamming capability made the engineered biosensor become practically feasible for on-site assessment of Hg^{2+} contamination in the real water samples.

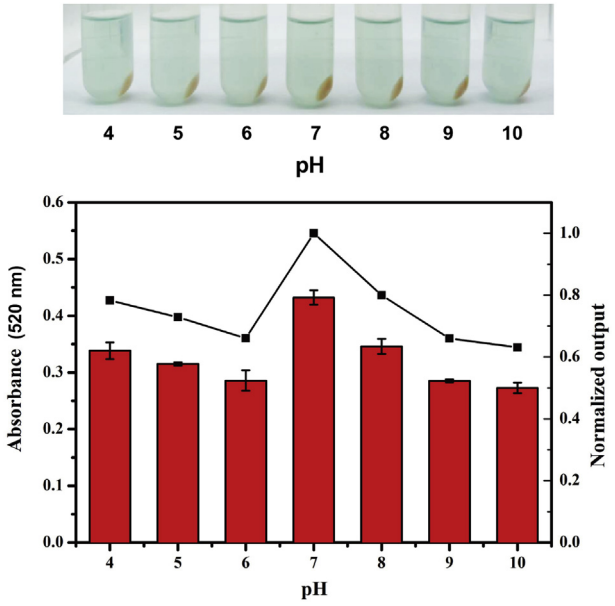

FIG. 4. The response of biosensor towards 100 nM Hg^{2+} under different pH values. All the data are the means of five independent experiments. Error bars correspond to the standard deviations.

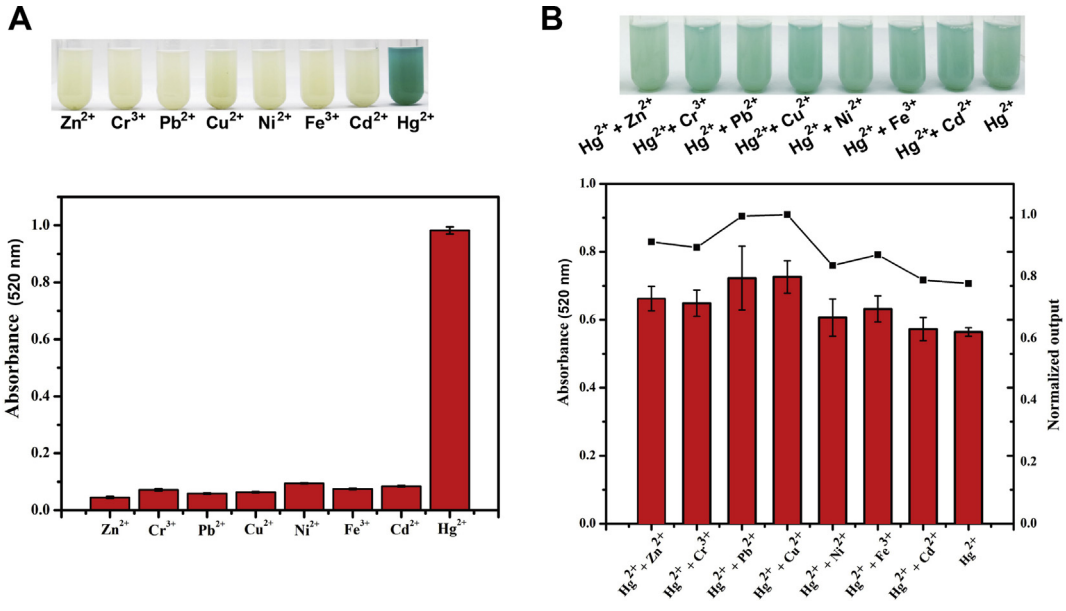

FIG. 3. The response of biosensor towards different metal ions. (A) The signals of the biosensor towards different metal ions at the concentration of 500 nM. (B) The signals of biosensor towards 100 nM Hg^{2+} in the presence of 100 nM Zn^{2+}, Cr^{3+}, Pb^{2+}, Cu^{2+}, Ni^{2+}, Fe^{3+}, and Cd^{2+}, respectively. All the data are the means of five independent experiments. Error bars correspond to the standard deviations.

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005

Effect of pH values on mercury sensing of the colorimetric biosensor The biosensor could perform function well in a relatively wide pH range (pH 4–10). As shown in Fig. 4, in the presence of Hg^{2+}, the biosensor showed the strongest detection signal at pH 7, and its signals were not greatly influenced with the varying pH values. Whether in acidic or alkaline range, the biosensor still showed obvious and intense detection signals towards Hg^{2+}. Based on previous researches, the wide pH working range of the biosensor was attributed to a series of periplasmic proteins and amino acid catabolism in microorganisms (30). It meant the whole-cell based biosensor was suitable for extensive detection with the aquatic systems having different pH values, which had great advantage over the most detection methods that only work in the specific conditions. For example, the chemosensors made from biological molecules, such as oligonucleotides, proteins, and DNA-protein complexes, always relied on mild physical environment for functional performance.

Application of colorimetric biosensor in environmental water detection As is well-known, the water systems in the natural environment include abundant microorganisms and the metabolites of them. Thus, majority of the chemosensors were unable to couple with the complex environment that greatly affected their performances. The signal molecule pyocyanin has extensive anti-bacterial and anti-fungal activities, which provides the protection of biosensor from the effects of microorganisms in the environmental water. As shown in Table 1, the developed biosensor could well evaluate the concentration of Hg^{2+} spiked in the lake water, which indicated that the present biosensor had the ability to resist kinds of interferences in the environmental water samples.

In contrast to traditional measurements, such as ICP-MS, AAS, and AFS, the present colorimetric biosensor could provide visual detection signal and didn't require complicated sample preparation, which was really appropriate for large-scale samples determination. Through the high-throughput determination, the developed biosensor had obviously economic and time advantages over the aforementioned traditional measurements. On the other hand, the developed biosensor was capable of reporting the fraction of Hg^{2+} that only interacts with the biota other than the total amount of mercury present in the samples. Thus, a direct estimation of toxicity for the bioavailable mercury in contaminated water can be preliminarily supplied by the present biosensor, whereas the accuracy of which needs further improvement to compare favorably with traditional measurements.

In conclusion, the simple, economic, visual, and effective platform for detecting Hg^{2+} was developed for preliminary estimation of the toxicity of bioavailable Hg^{2+}. This approach takes advantage of the fact that the cells can respond specifically to Hg^{2+} with high sensitivity via Hg^{2+}-dependent transcription factor MerR. The biosynthesis pathway of pyocyanin is also made best use of in the detection system, which offered a convenient manner for signal detection and strong capacity of resisting interferences generated by other microorganisms. Finally, thanks to the bacterial metabolism, the whole-cell biosensor also had an extensive working pH range. Based on above design philosophy, more biochemical systems can be engineered to meet the environmental sample detection requirements for heavy metal ions as well as other harmful chemicals.

TABLE 1. Detection of Hg^{2+} added in lake water by the developed biosensor.

Sample	Spiked (nM)	Detected (nM)	Recovery (%)	R.S.D. (%)
Lake water 1	25	24.82 ± 0.72	99.28	2.90
	50	46.32 ± 3.15	92.64	6.80
	100	113.32 ± 0.83	113.32	0.73
	250	258.87 ± 24.69	103.54	9.53
Lake water 2	25	31.05 ± 1.45	124.20	4.67
	50	52.37 ± 1.76	104.74	3.36
	100	91.08 ± 3.88	91.08	4.26
	250	253.66 ± 16.29	101.46	6.42
Lake water 3	25	25.08 ± 1.11	100.32	4.43
	50	50.23 ± 4.41	104.46	8.78
	100	102.03 ± 11.57	102.03	11.34
	250	292.46 ± 9.99	116.98	3.41

R.S.D. (%) means relative standard deviation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2019.08.005.

ACKNOWLEDGMENTS

This research was financially supported by National Natural Science Foundation of China (31800631), Natural Science Foundation of Guangxi Province (2018JJB120049), Middle-aged and Young Teachers' Basic Ability Promotion Project of Guangxi (2018KY0361), and BAGUI Scholar Program of Guangxi Province of China.

References

1. **Harris, H. H., Pickering, I. J., and George, G. N.:** The chemical form of mercury in fish, Science, **301**, 1203 (2003).
2. **Manceau, A., Enescu, M., Simionovici, A., Lanson, M., Gonzalez-Rey, M., Rovezzi, M., Tucoulou, R., Glatzel, P., Nagy, K. L., and Bourdineaud, J.-P.:** Chemical forms of mercury in human hair reveal sources of exposure, Environ. Sci. Technol., **50**, 10721–10729 (2016).
3. **Prüss-Ustün, A., Vickers, C., Haefliger, P., and Bertollini, R.:** Knowns and unknowns on burden of disease due to chemicals: a systematic review, Environ. Health, **10**, 9 (2011).
4. **Bridges, C. C. and Zalups, R. K.:** Mechanisms involved in the transport of mercuric ions in target tissues, Arch. Toxicol., **91**, 63–81 (2017).
5. **Gao, Y., Shi, Z., Long, Z., Wu, P., Zheng, C., and Hou, X.:** Determination and speciation of mercury in environmental and biological samples by analytical atomic spectrometry, Microchem. J., **103**, 1–14 (2012).
6. **Nolan, E. M. and Lippard, S. J.:** Tools and tactics for the optical detection of mercuric ion, Chem. Rev., **108**, 3443–3480 (2008).
7. **Zhang, R. and Chen, W.:** Nitrogen-doped carbon quantum dots: facile synthesis and application as a "turn-off" fluorescent probe for detection of Hg^{2+} ions, Biosens. Bioelectron., **55**, 83–90 (2014).
8. **Guo, Y., Zhang, L., Zhang, S., Yang, Y., Chen, X., and Zhang, M.:** Fluorescent carbon nanoparticles for the fluorescent detection of metal ions, Biosens. Bioelectron., **63**, 61–71 (2015).
9. **Chen, G., Guo, Z., Zeng, G., and Tang, L.:** Fluorescent and colorimetric sensors for environmental mercury detection, Analyst, **140**, 5400–5443 (2015).
10. **Zhou, Y., Tang, L., Zeng, G., Zhang, C., Zhang, Y., and Xie, X.:** Current progress in biosensors for heavy metal ions based on DNAzymes/DNA molecules functionalized nanostructures: a review, Sens. Actuators B Chem., **223**, 280–294 (2016).
11. **Gumpu, M. B., Sethuraman, S., Krishnan, U. M., and Rayappan, J. B. B.:** A review on detection of heavy metal ions in water–an electrochemical approach, Sens. Actuators B Chem., **213**, 515–533 (2015).
12. **Kim, H. J., Jeong, H., and Lee, S. J.:** Synthetic biology for microbial heavy metal biosensors, Anal. Bioanal. Chem., **410**, 1191–1203 (2018).
13. **Wei, W., Liu, X., Sun, P., Wang, X., Zhu, H., Hong, M., Mao, Z.-W., and Zhao, J.:** Simple whole-cell biodetection and bioremediation of heavy metals based on an engineered lead-specific operon, Environ. Sci. Technol., **48**, 3363–3371 (2014).
14. **Wei, W., Zhu, T., Wang, Y., Yang, H., Hao, Z., Chen, P. R., and Zhao, J.:** Engineering a gold-specific regulon for cell-based visual detection and recovery of gold, Chem. Sci., **3**, 1780–1784 (2012).
15. **Cai, S., Shen, Y., Zou, Y., Sun, P., Wei, W., Zhao, J., and Zhang, C.:** Engineering highly sensitive whole-cell mercury biosensors based on positive feedback loops from quorum-sensing systems, Analyst, **143**, 630–634 (2018).
16. **Jouanneau, S., Durand, M.-J., Courcoux, P., Blusseau, T., and Thouand, G.:** Improvement of the identification of four heavy metals in environmental samples by using predictive decision tree models coupled with a set of five bioluminescent bacteria, Environ. Sci. Technol., **45**, 2925–2931 (2011).
17. **Guo, K. H., Chen, P. H., Lin, C., Chen, C. F., Lee, I. R., and Yeh, Y. C.:** Determination of gold ions in human urine using genetically engineered microorganisms on a paper device, ACS Sens., **3**, 744–748 (2018).
18. **Yin, K., Lv, M., Wang, Q. N., Wu, Y. X., Liao, C. Y., Zhang, W. W., and Chen, L. X.:** Simultaneous bioremediation and biodetection of mercury ion through surface display of carboxylesterase E2 from *Pseudomonas aeruginosa* PA1, Water Res., **103**, 383–390 (2016).
19. **Gui, Q., Lawson, T., Shan, S., Yan, L., and Liu, Y.:** The application of whole cell-based biosensors for use in environmental analysis and in medical diagnostics, Sensors, **17**, 1623 (2017).

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005

20. **Greenhagen, B. T., Shi, K., Robinson, H., Gamage, S., Bera, A. K., Ladner, J. E., and Parsons, J. F.:** Crystal structure of the pyocyanin biosynthetic protein PhzS, Biochemistry, **47**, 5281–5289 (2008).
21. **Mavrodi, D. V., Bonsall, R. F., Delaney, S. M., Soule, M. J., Phillips, G., and Thomashow, L. S.:** Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1, J. Bacteriol., **183**, 6454–6465 (2001).
22. **Wang, D., Huang, S., Liu, P., Liu, X., He, Y., Chen, W., Hu, Q., Wei, T., Gan, J., Ma, J., and Chen, H.:** Structural analysis of the Hg(II)-regulatory protein *Tn501* MerR from *Pseudomonas aeruginosa*, Sci. Rep., **6**, 33391 (2016).
23. **Essar, D. W., Eberly, L., Hadero, A., and Crawford, I. P.:** Identification and characterization of genes for a second anthranilate synthase in *Pseudomonas aeruginosa*: interchangeability of the two anthranilate synthases and evolutionary implications, J. Bacteriol., **172**, 884–900 (1990).
24. **Brown, N. L., Stoyanov, J. V., Kidd, S. P., and Hobman, J. L.:** The MerR family of transcriptional regulators, FEMS Microbiol. Rev., **27**, 145–163 (2003).
25. **Watson, D., Macdermot, J., Wilson, R., Cole, P. J., and Taylor, G. W.:** Purification and structural analysis of pyocyanin and 1-hydroxyphenazine, Eur. J. Biochem., **159**, 309–313 (1986).
26. **Ding, S.-Y., Dong, M., Wang, Y.-W., Chen, Y.-T., Wang, H.-Z., Su, C.-Y., and Wang, W.:** Thioether-based fluorescent covalent organic framework for selective detection and facile removal of mercury(II), J. Am. Chem. Soc., **138**, 3031–3037 (2016).
27. **Nie, K., Dong, B., Shi, H., Liu, Z., and Liang, B.:** Diketopyrrolopyrrole amphiphile-based micelle-like fluorescent nanoparticles for selective and sensitive detection of mercury(II) ions in water, Anal. Chem., **89**, 2928–2936 (2017).
28. **Stocker, J., Balluch, D., Gsell, M., Harms, H., Feliciano, J., Daunert, S., Malik, K. A., and van der Meer, J. R.:** Development of a set of simple bacterial biosensors for quantitative and rapid measurements of arsenite and arsenate in potable water, Environ. Sci. Technol., **37**, 4743–4750 (2003).
29. **Cerminati, S., Soncini, F. C., and Checa, S. K.:** A sensitive whole-cell biosensor for the simultaneous detection of a broad-spectrum of toxic heavy metal ions, Chem. Commun., **51**, 5917–5920 (2015).
30. **Stancik, L. M., Stancik, D. M., Schmidt, B., Barnhart, D. M., Yoncheva, Y. N., and Slonczewski, J. L.:** pH-dependent expression of periplasmic proteins and amino acid catabolism in *Escherichia coli*, J. Bacteriol., **184**, 4246–4258 (2002).

Please cite this article as: Wang, D et al., Visual detection of Hg^{2+} by manipulation of pyocyanin biosynthesis through the Hg^{2+}-dependent transcriptional activator MerR in microbial cells, J. Biosci. Bioeng., https://doi.org/10.1016/j.jbiosc.2019.08.005