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Feedback regulation mode of gene circuits directly affects the detection range and sensitivity of lead and mercury microbial biosensors

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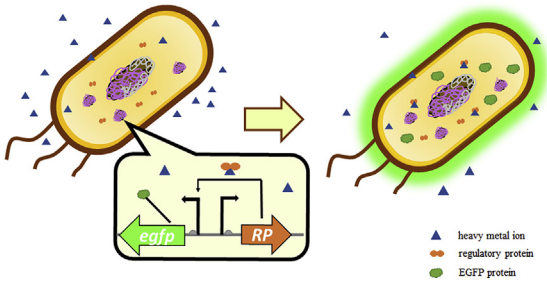
HIGHLIGHTS

- A general instruction for the efficient and targeted design of heavy metal microbial biosensors is provided.
- Three basic models of lead and mercury detection circuits, namely feedback, semi-coupled and uncoupled, were constructed.
- The uncoupled model showed better sensitivity for both lead (50 nM - 10 μM) and mercury (1 nM–50 nM).
- Feedback circuits had a wider detection range for mercury (10 nM - 7.5 μM).
- This study established a novel and simple pre-treatment method for biosensors in milk and sewage.

GRAPHICAL ABSTRACT



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ABSTRACT

Whole cell biosensors offer high potential for the detection of heavy metals in a manner that is simple, rapid and low-cost. However, previous researchers have paid little attention to the impacts of construction models on the performance of these biosensors, thereby limiting the achievement of rational design and the optimization of detection characteristics. Herein, for the first time, three basic models of lead and mercury detection circuits, namely feedback coupled, uncoupled and semi-coupled models, have been constructed and compared to explore the effects of uncoupling the topology of sensing circuits on the reporter signals. The results demonstrated that the uncoupled model had better sensitivity for both lead (50 nM) and mercury (1 nM), while the feedback coupled circuits had a wider detection range for mercury (10 nM - 7.5 μM). Introducing the semi-coupled model into the comparison revealed that both the type and location of promoters for regulatory protein genes were key factors for sensitivity. Moreover, the detection characteristics of the uncoupled biosensors were robust, as conditions such as induction time, the concentration of microbial cells, and the concentration of antibiotics had little interference on the performance of the microbial biosensors. This study also established a novel and

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simple pre-treatment method for sample detection by biosensors. When the uncoupled microbial biosensor was put into practice, the concentration levels of mercury in milk and lead in sewage were determined quickly and accurately. Our study, therefore, provides a strategy for the rational design of whole cell heavy metal biosensors and has developed the potential of their application.

1. Introduction

In recent decades, the expansion of industrialization, coal mining and wastewater discharge have led to a dramatic increase of heavy metals in soil, water and atmosphere [1]. As heavy metals cannot be degraded, they accumulate in the environment causing severe harm to the ecosystem and to human bodies [2]. Heavy metals in the environment access the human body through the uptake of food and water, as well as through respiratory inhalation and skin contact, with obvious toxic effects even at low concentrations. Their high cumulative effect can lead to chronic poisoning and long-term effects [3], which is why it is critical that these heavy metals are detected in order to avoid exposure to such harm. However, current methods for the chemical analyses of heavy metals are either expensive or logistically difficult to perform [4], are time-consuming and use costly instruments [5], or require complex pre-treatments and large sample volumes, all of which limits their application in situ [6]. There is, therefore, an urgent need for alternative simple, portable and accurate detection methods that do not require large scale equipment. Biosensors are hot spots in this focus for heavy metal determinations. Functional nucleic acid detection technology, for example, meets the requirements of being rapid and accurate when detecting mercury and lead. The nucleic acid probes form T-Hg^{2+}-T in the presence of mercury ions and the DNAzyme fractures under the action of Pb^{2+}. Although these methods are ingenious, they lack universality for other metals and, furthermore, cell-free biosensors present problems with stability.

A microbial whole cell biosensor (WCB) is a novel device for the qualitative and quantitative detection of physical, chemical and biophysical molecules through the construction of genetic circuits in microorganisms [7]. The genetic circuits consist of the reporter genes and the transcription factors that respond to various signals, thus transforming biological responses into measurable signals [8]. The WCBs are easy to construct, detect rapidly and can be employed without the limitations of detection environments. Consequently, they have been widely used in the analysis of heavy metals in mediums such as water [9], soil [10], air [11], food-stuffs [12], urine [13,14] and blood serum [15,16]. However, the sensitivity and performance of WCBs in different samples still need to be improved.

Genetic circuits of biosensors for heavy metals detection are typically based on the resistance systems of bacteria, such as the mercury resistance system encoded by the *mer* operon on transposons Tn501 from *Pseudomonas aeruginosa* [17] and Tn21 from *Shigella flexneri* R100 plasmid [18]. This resistance system is homeostatically regulated by the MerR activator at the transcription level [17,19]. MerR is a Hg (II)-responsive transcription factor with a high affinity for the DNA binding site, which is positioned between −35 and −10 elements of the *mer* promoter as either an activator or a repressor. MerR regulatory protein can allosterically initiate the transcription of genes in the operon, including the *merR* gene itself in the presence of mercury [20], by remodeling the promoter structure. At first, to construct mercury biosensors, most researchers used the feedback coupled circuit in which the reporter gene was in the *mer* operon under the control of Pmer (regulated by MerR protein) [21–27]. However, since the feedback loop retains the original circuit of the *mer* operon, the reporter gene has a basic expression which leads to a disturbingly high background value. Recently, researchers have developed different mercury biosensors in which the expression of the *merR* is uncoupled from its feedback loop, however no research has been conducted to systematically compare these two kinds of circuits and establish the mechanisms therein.

The *pbr* operon in *Cupriavidus metallidurans* CH34 encodes a bacterial Pb (II) specific resistance system with a strong specificity [28,29]. Like MerR, PbrR binds to the DNA site between the −35 to −10 elements of Ppbr and allosterically activates the expression of structure genes and itself in the presence of Pb (II) (A et al., 2003; [30–32]. Until now, lead biosensors have mainly relied on the feedback loop, with different reporter genes [21,33]. The uncoupled circuit for lead detection has not been studied.

To ascertain the effects of the gene circuits, we constructed three kinds of biosensors each for lead and mercury. The objectives of the current work were to explore the differences between the feedback coupled, semi-coupled and uncoupled circuits on enhanced green fluorescent protein (EGFP) expressions and sensitivity. We determined that uncoupling could have important benefits for eGFP output and result in adjustable maximum reporter levels. In addition, to verify the applicability of our biosensors, the uncoupled circuits were practically applied in lead and mercury detection in different samples. The results showed that our novel biosensors can work normally in water, milk and sewage samples.

2. Experimental sections

2.1. Bacterial strain, plasmids, and oligonucleotides

Escherichia coli DH5α was used as receptor cells for all the plasmids in this study. Plasmids and oligonucleotides used in this study are listed in Table S1. Oligonucleotide synthesis and plasmid sequencing were conducted by Ruibio Biotech (Beijing, China).

2.2. Construction of Plasmids and biosensors

A plasmid named pMetalBasicGreen was first constructed by redesigning the restriction sites of commercialized plasmid pENTR/D-TOPO. All plasmids used in this study were constructed by inserting different circuits into pMetalBasicGreen. For the construction of feedback coupled circuits, the *merR* gene and bidirectional promoter P*mer* from *mer* operon of *Pseudomonas aeruginosa* were kept and the downstream mercury resistance genes *merTPAD* on the other strand were replaced by *egfp* gene. Similarily, *pbrR* gene and promoter P*pbr* from *pbr* operon of *Cupriavidus metallidurans* were kept and lead resistance genes *pbrABCD* were replaced by *egfp* gene. For the construction of uncoupled circuits, the expression of *egfp* gene was still controlled by promoter P*mer* or P*pbr*, and the expression of *merR* or *pbrR* gene was controlled by a moderate constitutive promoter P558. For the construction of semi-couple circuits, the expression of *egfp* gene was still controlled by promoter P*mer* or P*pbr*, and the expression of *egfp* gene was still

controlled by another promoter P*mer* or P*pbr*. These six circuits were respectively inserted into pMetalBasicGreen to form plasmid pMerRfeedback, pPbrRfeedback, pMerRuncoupled, pPbrRuncoupled, pMerRsemi, and pPbrRsemi. A heat shock procedure was employed for the transformation of these plasmids into *E. coli* DH5α strains to form the biosensor merR-egfp, pbrR-egfp, merR558, pbrR558, merRffk, pbrRffk respectively (Table S2). LB medium comprising 10 g/L Tryptone (OXOID, Basingstoke, UK), 5 g/L yeast extract (OXOID, Basingstoke, UK), 5 g/L NaCl (BEIJING SHIJI, Beijing, China) and 15 g/L agar (Biotopped, Beijing, China) (in solid medium) was used in the cultivation or isolation of pure cultures. For positive selection of the biosensor cells, kanamycin solution (BIOSHARP, Anhui, China) was added to LB media at the concentration of 50 μg/mL. The biosensors were stored at −80 °C until use.

2.3. Biosensor preparation and fluorescence measurement

The biosensors were first activated by overnight growth in LB broth with Kanamycin. Before the bioassay, the activated biosensors were diluted into fresh LB broth containing Kanamycin and incubated at 37 °C and 220 rpm until the OD600 reached approximately 0.6. Then 5 mL biosensor cultures, 5 mL fresh LB broth with Kanamycin, and 100 μL of heavy metal solution (0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 5 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 2.5 μM, 5 μM, 7.5 μM, 10 μM, 20 μM, 40 μM, 60 μM, 80 μM, or 100 μM Hg(II), or 10 nM, 50 nM, 100 nM, 500 nM, 1 μM, 2.5 μM, 5 μM, 7.5 μM, 10 μM, 20 μM, 40 μM, 60 μM, 80 μM, or 100 μM Pb(II) respectively) were mixed in 50 mL flasks and incubated at 37 °C and 220 rpm for 2 h 200 μL of cultures were taken for the measurement of OD600. Another 2 mL of the cultures were taken and centrifuged at 10000×*g*, and sediment was suspended again with 200 μL of 0.9% saline. The fluorescence intensity of this solution was measured using a full-wavelength spectral scanner (Thermo Fisher, Waltham, USA) with the excitation wavelength was 488 nm, and the emission wavelength was 507 nm. The fluorescence measurements were expressed as relative fluorescence, calculated as the fluorescence intensity divided by OD600. To optimize the initial cell density and incubation time, 5 mL biosensor cultures with OD600 to be 0.4 or 0.6 were mixed with 5 mL fresh LB broth and 100 μL of heavy metal solution, and the fluorescence intensity was measured after incubation for 1 h, 2 h, or 3 h. To test the robustness of the biosensor in different condition, different concentration of kanamycin (25, 50, or 75 μg/mL) or 100 nM of different interfering ions was respectively added into the detection system.

2.4. Heavy metal ions detection in samples

To test the performance of biosensor merR558 in milk samples, two detection systems were compared. In the first system, 5 mL biosensor cultures (OD600 = 0.6) and 5 mL milk samples containing mercury ions were mixed in 50 mL flasks and incubated at 37 °C and 220 rpm for 2 h. In the second system, 5 mL biosensor cultures (OD600 = 0.6), 5 mL fresh LB broth with Kanamycin, and 100 μL milk samples containing mercury ions were mixed in 50 mL flasks and incubated at 37 °C and 220 rpm for 2 h. For the use of biosensor pbrR558 in lead detection of sewage samples, 5 mL biosensor cultures (OD600 = 0.6), 5 mL fresh LB broth with Kanamycin, and 100 μL sewage samples containing lead ions were mixed in 50 mL flasks and incubated at 37 °C and 220 rpm for 2 h. The measurement and calculation of the relative fluorescence were the same as described in the previous section.

2.5. Data analysis

Each procedure was repeated three times, and all values were expressed as the arithmetic mean ± standard deviation (S.D.). The data was analyzed using a one-way analysis of variance (ANOVA) followed by a *t*-test. The significant analysis of the difference between linear detection curves of biosensors was conducted by covariance analysis using SPSS statistics v19. The P values of the differences of the slopes and intercepts were calculated.

3. Results and discussion

3.1. Comparison of the performance of the feedback and uncoupled circuits

In the construction of the best mercury and lead whole cell biosensors, certain regularities were noted in the construction modes. Using the *merR* operon genes from *Pseudomonas aeruginosa* and the *pbrR* operon genes from *Cupriavidus metallidurans*, three kinds of circuits were designed, as shown in Fig. 1. In the feedback circuits, both the *egfp* and *merR/pbrR* gene were under the control of the promoter P*mer* or P*pbr* (Fig. 1A), which means the expression of the regulatory protein MerR and PbrR were controled by themselves and their expression levels were dynamic. Uncoupled circuits were subsequently constructed in which the expression of MerR/PbrR was separated from its feedback control and their expression levels kept relatively constant (Fig. 1B).

The performances of the feedback and uncoupled biosensors were compared through induction by metal ions. For mercury feedback biosensor merR-egfp, the relative fluorescence intensity of eGFP showed a linear relationship with the concentration of exposed Hg^{2+} over a range of 10 nM-7.5 μM (Fig. 2A). When the concentration levels rose above 40 μM, as shown in Fig. S1, the engineered *E. coli* was no longer able to express fluorescent protein and its growth was also inhibited. This could be due to the toxin of the heavy metals, which harms the metabolism and inhibits the growth of microbes. In the uncoupled biosensor merR558, the concentrations of metal ions were the only variate in the detection process and it was conjectured that the sensitivity might increase

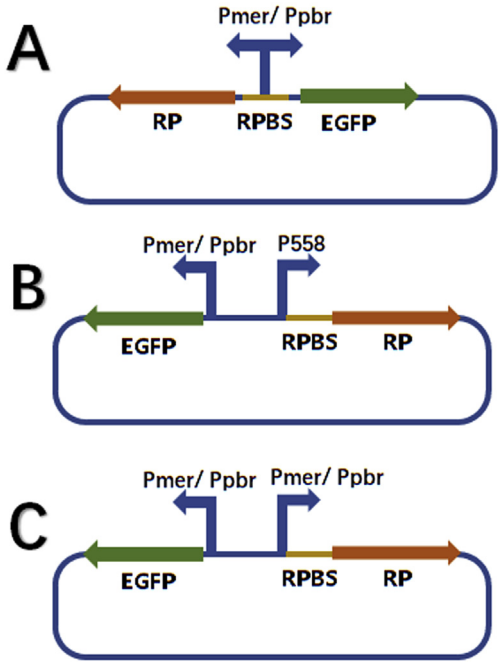

Fig. 1. Construction circuits of the engineered *E. coli* biosensors. A: Elements building the feedback construct; B: Elements building the uncoupled construct; C: Elements building the semi-coupled construct.

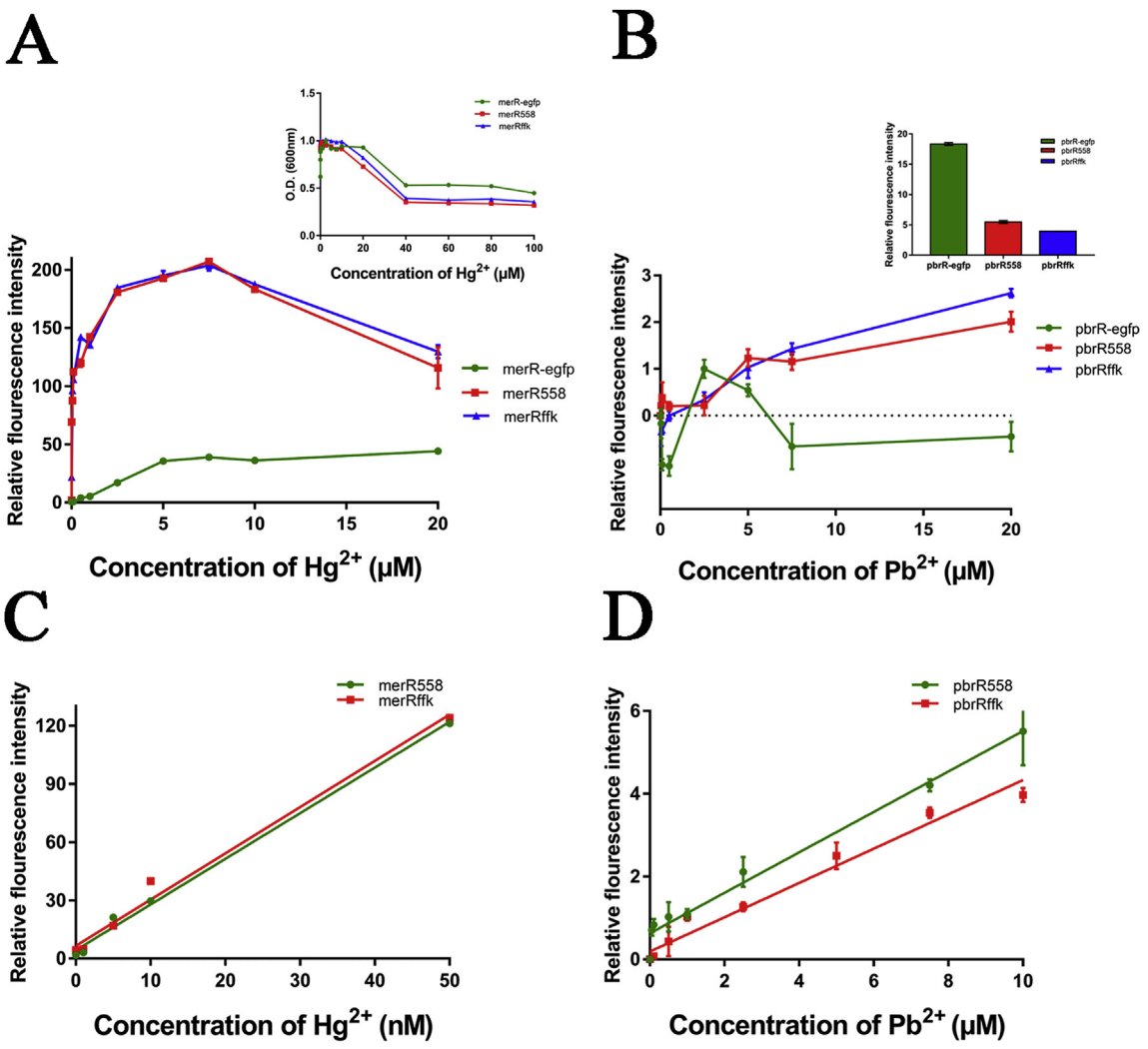

Fig. 2. A: eGFP fluorescence of biosensor merR558, merRffk, merR-*egfp* plasmids as a function of mercury exposure, measured 120 min after induction; the embedded panel indicated the OD600 values of these biosensor after incubation with mercury. B: eGFP fluorescence of biosensor pbrR558, pbrRffk, pbrR-*egfp* as a function of lead exposure, measured 120 min after induction; the embedded panel indicated the background fluorescence intensity of these biosensor after incubation with no lead water. C: eGFP fluorescence of merR558, merRffk plasmids as a function of mercury exposure, measured 120 min after induction; D: eGFP fluorescence of biosensor pbrR558, pbrRffk plasmids as a function of lead exposure, measured 120 min after induction.

by removing the interference of expression level of regulatory protein. Our results confirmed the above conjecture. The uncoupled biosensor merR558 brought about a higher sensitivity but a narrower linear range (1–100 nM) than the feedback biosensor (Fig. 2A). For lead biosensor, when treated with gradient-diluted lead ions solutions, the production of eGFP in the feedback biosensor pbrR-egfp was low and there was no regularity between the eGFP expression and Pb^{2+} concentrations (Fig. 2B). Inversely, the uncoupled lead biosensor pbrR558 had better linearity and the detection range was between 50 nM and 10 μM, which is a significant improvement on the performance of the feedback circuit (Fig. S2). The different results between merR biosensor and pbrR biosensor might be due to the different promoter strength between P*pbr* and P*mer*. Furthermore, in the feedback biosensor pbrR-egfp, a slight increase in the background production of eGFP was observed. This might be the result of the lower basic expression of regulatory proteins in origin operon, which resulted in relatively higher leakage expression of eGFP.

Compared with the mercury and lead biosensors reported in previous studies, our uncoupled biosensors showed obvious improvements in sensitivity and linearity. For example, in a recently reported lead biosensor based on the feedback *pbrR* circuit, the limit of detection (LOD) was at the level of 5 μg/ml (about 25 μM) in *E. coli* DH5α receptor strain and 0.2 μg/ml (about 1 μM) in *Pseudomonas aeruginosa* PAO1 receptor strain [33]. By the using of uncoupled circuit, the biosensor pbrR558 in our research showed a LOD of 50 nM, which was an improvement of one or two order of magnitude. In a recent study on mercury biosensors based on the feedback *merR* circuits, the biosensor showed no detection capability at the range of 1 nM–100 nM unless the introduction of quorum sensing systems for signal amplification [34]. However, combination of different systems affected the linearity of detection. Our uncoupled biosensor merR558 achieved the mercury detection at the range of 1–100 nM without introduction of signal amplification system, and thus with better linearity of detection.

3.2. The semi-coupled circuits reveal factors that influence the sensitivity of biosensors

The above results indicate that uncoupled circuits are more sensitive during detection, although their detection range is somewhat narrow. It was not easy to find an explanation for this

phenomenon, because the promoter of regulatory protein and the construction manner were both changed from the feedback circuit. Therefore, to investigate the effect of promoters in optimizing the microbial biosensor, the semi-coupled circuits were created by substituting the inducible promoter containing MerR/PbrR binding site for the constitutive promoter 558 (Fig. 1C), which is to say, in semi-couple circuits, the expression of *egfp* and the expression of *merR* or *pbrR* gene were connected in regulation mechanism but uncoupled in steric hindrance.

Treated with different concentrations of Hg ions, the semi-coupled biosensor merRffk produced a similar performance to the uncoupled biosensor merR558 in terms of sensitivity, background value and linear range for the detection of lead and mercury (Fig. 2C). The slope and intercept of the linear detection curves of merR558 and merRffk showed no significant differences ($P_{slope} = 0.521$, $P_{intercept} = 0.706$). A significant difference was noted between the intercepts of linear detection curves of the lead biosensor pbrRffk and biosensor pbrR558, though the slopes of linear detection curves were still very similar (Fig. 2D, $P_{slope} = 0.177$, $P_{intercept}<0.01$). It was probably because their eGFP expressions were less, thus producing slight fluctuations that can cause significant change. The results indicate that the type and location of promoter are both key factors in the construction of a microbial biosensor and play a major role in increasing sensitivity.

3.3. Detection characteristics of uncoupled biosensors were robust as detection conditions changed

Previous studies have shown that the optimal reaction conditions differ between biosensors. To ascertain these optimum conditions, various induction times (1 h, 2 h and 3 h) and optical densities of biosensors ($OD_{600} = 0.4$, $OD_{600} = 0.6$) (Fig. 3A and B) were investigated, keeping other conditions consistent.

The linear results were found to be better when the concentration of biosensor cells was $OD_{600} = 0.4$ than 0.6 in lead biosensor pbrR558, while there was no obvious difference in mercury biosensor merR558. On the other hand, a shorter induction time led to a lower expression of fluorescent proteins. In mercury biosensor merR558, the best linear range took place after 3 h of induction, while the lead biosensor pbrR558 was suitable after 2 h induction, although the differences between these conditions were slight. Longer incubation time than 3 h led to higher fluorescence intensity of the biosensor but bad linear characteristics of the detection (data not shown). Further more, as a detection method, cutting down the detection time was very important. Thus the situation of longer incubation time was not deeply studied in this research.

On the other hand, since the resistance gene was also inserted into the plasmid for selection, we suspected the concentration of kanamycin in the culture medium to be an important influencing factor. It was conjectured that, as kanamycin concentrations increased, the number of copies of plasmids in the bacteria might increase, possibly accompanied by an increase in the regulatory protein concentration, while the amount of regulatory proteins in the cell would control the sensitivity.

We tested our theory by setting three different kanamycin concentrations and keeping the other variables consistent. However, no significant changes were observed and the eGFP output at different concentrations of metal ions were similar (Fig. 3C and D). This suggests that the increase of plasmid copies not only increases the amount of intracellular regulatory proteins, but also increases the quantity of binding sites. The proportions between regulatory protein and binding site were basically stable, producing no significant changes in sensitivity.

In order to confirm the effects of interfering substances, measurement of the analyte metal under the presence of other heavy metal ions are tested. As shown in Fig. 3E and F, the measurements of mercury ion by uncoupled biosensor merR558 were only affected by cadmium ion. The fluorescence intensity of biosensor merR558 incubated with 100 nm mercury ion and 100 nm cadmium ion showed a slight but significant ($P < 0.05$) decrese comparing with that incubated with only 100 nm mercury ion. Other ions including lead, copper, silver, zinc, cobalt, nickel, iron had no interfering effects on the biosensor merR558. For the detection of lead ion by biosensor pbrR558, all the test ions alone had no interfering effects. These results confirmed the robustness and anti-interference ability of our biosensors. However, as an extreme case, a mix of the above interfering ions showed significant influence on both biosensor merR558 and biosensor pbrR558. Thus the robustness and anti-interference ability of our biosensors were within a certain limit.

3.4. The applications of the uncoupled microbial biosensors in milk and sewage samples

Subsequent to the study of robustness of the uncoupled biosensors in different reaction conditions, the biosensors' performance was verified in terms of application. Mercury pollution is common in dairy products, while lead pollution is common in sewage. Therefore, two situations, mercury in milk and lead in sewage, were designed to verify the capacity of uncoupled biosensors when detecting the not-one-single-component samples.

In the determination of mercury ions in milk, the previous system was modified by replacing 5 mL culture medium with milk containing mercury ions. The results obtained by this method were not accurate as the excessive addition of milk increased the optical density (OD) value, thus reducing the relative fluorescence intensity. Therefore, the calculated concentration of mercury ions in the milk samples was about half of the actual concentration. Consequently, an alternative method was devised with which to experiment with milk and sewage detections.

A gradient dilution of mercury ions was added into ultrapure water (standard curve) and milk. Next, 100 μL milk was added into the 10 mL system. After inducting the biosensor merR558 for 2 h, OD_{600} and fluorescence intensity were determined simultaneously. As shown in Fig. 4A, the green curve (milk sample) provided similar results to the red standard curve (water sample), indicating that the uncoupled mercury biosensor had excellent detection capability in milk ($P_{slope} = 0.994$, $P_{intercept} = 0.936$). In China, the limit of mercury content in milk is 0.01 mg/kg and the determination requires that 2 g samples be dissolved in 50 mL water, with the final concentration approximately 2 nM. The detection range of the novel biosensor in this study is 1–50 nM in milk samples, thus adequately meeting Chinese standard so that it can meet the requirements of national standards. And the centrifugation in pre-treatment does not interfere with experimental results.

Similarly, after adding lead ions into sewage and ultra-pure water simultaneously, the detection results of these two situations by biosensor pbrR558 were compared. As the results showed (Fig. 4B), the biosensor pbrR558 preserved its lead detection capability in sewage environment (R^2 in ultra-pure water was 0.9942, R^2 in sewage was 0.9882), though the characteristics of detection curve changed ($P_{slope}<0.01$, $P_{intercept}<0.01$). In China, the limitation of lead ions in sewage discharge standard is 4.8 μM, which is within the detection range of the biosensors. Impurities in sewage would affect the clarity of the solution, increasing the OD value and, consequently, decreasing the relative fluorescence intensity. However, this effect can be mitigated, as long as the standard solution conditions are consistent with the sample.

Meanwhile, the results showed that the high concentration

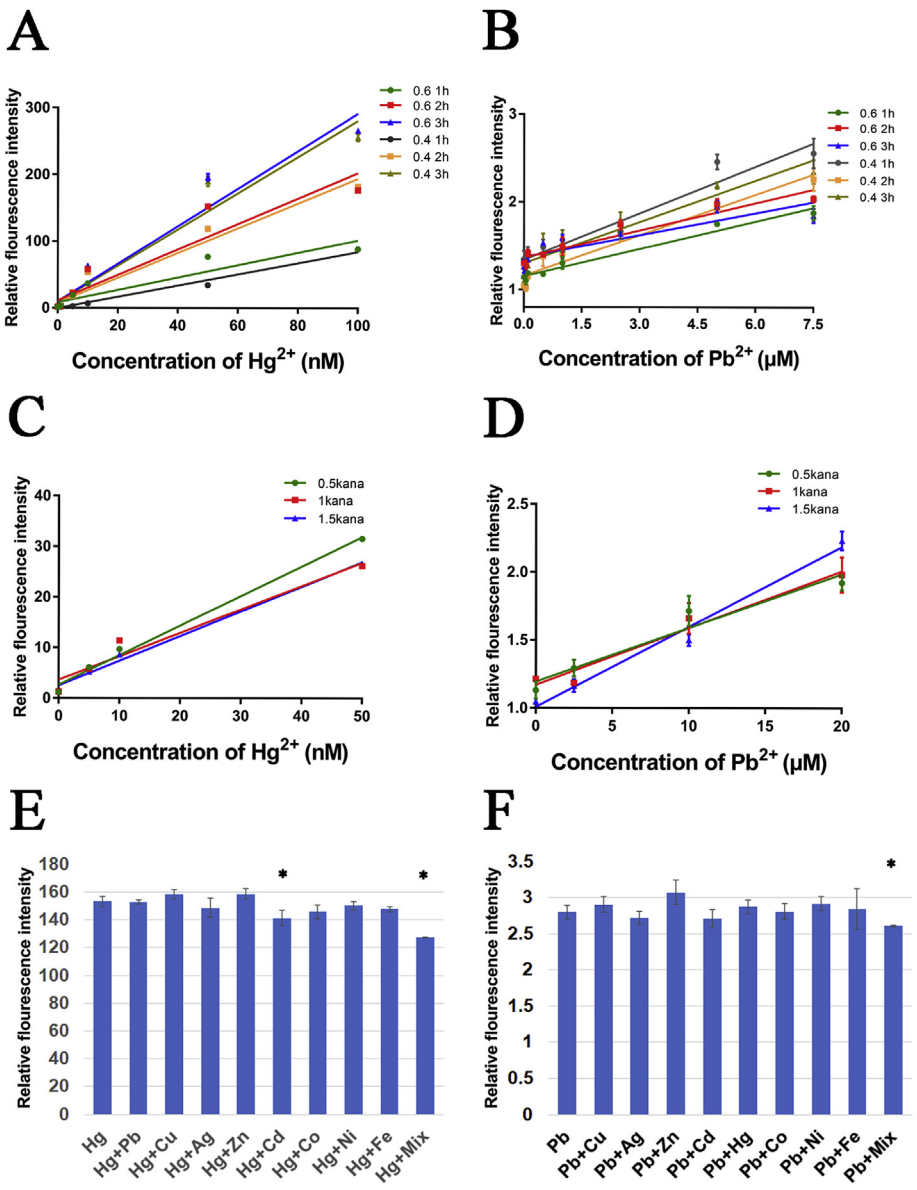

Fig. 3. A:eGFP fluorescence of different treatments as a function of mercury exposure, measured 120 min after induction; B: eGFP fluorescence of different treatments as a function of lead exposure, measured 120 min after induction; C: eGFP fluorescence of different concentrations of kanamycin as a function of mercury exposure, measured 120 min after induction; 0.5 kana, 1 kana, and 1.5 kana indicated the concentration of 25, 50, and 75 μg/mL kanamycin. D: eGFP fluorescence of different concentrations of kanamycin as a function of lead exposure, measured 120 min after induction. E: the eGFP fluorescence of biosensors merR558 incubated with 100 nM mercury ion alone, or 100 nM mercury and 100 nM interfering ions, or 100 nM mercury and metal ion mix (mix of 10 nM Pb, Cu, Ag, Zn, Cd, Co, Ni, Fe). The asterisk indicated significant difference compared with the fluorescence of biosensor incubated with 100 nM mercury ion alone. F: the eGFP fluorescence of biosensors pbrR558 incubated with 100 nM lead ion alone or 100 nM mercury and 100 nM interfering ions. The asterisk indicated significant difference compared with the fluorescence of biosensor incubated with 100 nM lead ion alone.

levels of eGFP could be observed by the naked eye. Hence, an experiment was designed in which mercury ions solutions of different concentrations were added to a solid medium and the biosensor merR558 were inoculated in a specific shape. After 12 h, the colonies with high ion concentrations (above 50 μM) became green, a phenomenon not observed in those with low levels of mercury ions (Fig. 5). This result verified that quantitative detection of microbial biosensors by the naked eye can be achieved through an adjustment in the expression levels of fluorescent proteins.

These experiment results verified the outstanding detection capacity of our feedback biosensors when applied into various samples. Moreover, the pre-treatment we established can rapidly and effectively remove interference factors in the sample, and support the biosensors in achieving accurate measurements.

In the practical application of the microbial biosensors, the pretreatment process was kept to a minimum. The novel pretreatment process contributes by reducing the detection time and also maintains the concentration level of ions in samples. The results indicate that the biosensor has practical application capacities, which are accurate and less time-consuming. However, at present, the microbial biosensors are still weak in stability and their performance is not consistent across different detection situations. With the development of science and technology and the deepening of research, the performance of microbial sensors will be perfected and the range of applications will become increasingly extensive.

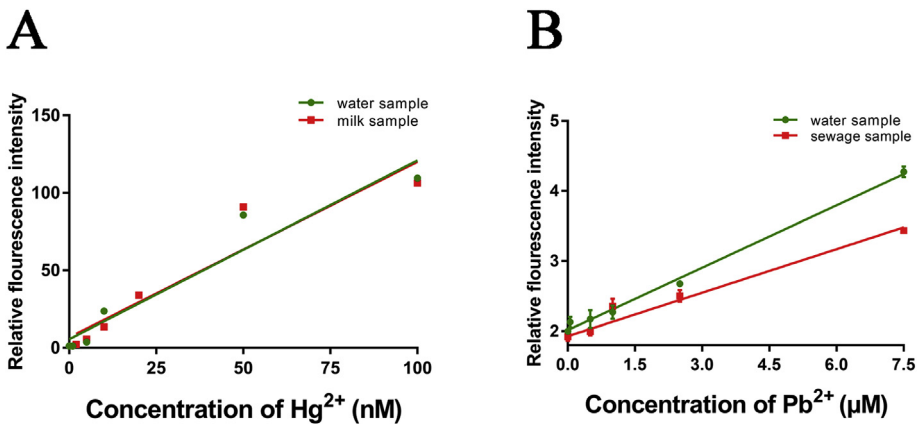

Fig. 4. A: eGFP fluorescence of uncoupled biosensor merR558 as a function of mercury exposure in milk, measured 120 min after induction; B: eGFP fluorescence of uncoupled biosensor pbrR558 as a function of lead exposure in sewage, measured 120 min after induction.

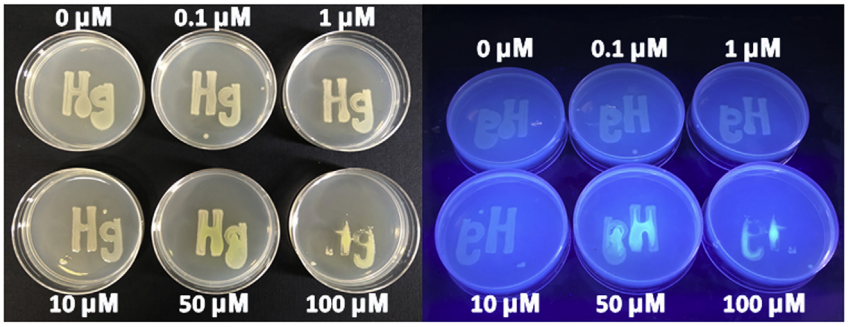

Fig. 5. eGFP fluorescence of uncoupled mercury biosensors colonies in the solid LB medium with different concentrations of mercury ions. The photographs were captured under naked eye (left) and UV light (right).

4. Conclusion

This work provides a general instruction to achieve efficient and targeted design of heavy metal microbial biosensors. Generally, the uncoupled biosensors provide a highly sensitive detection performance, while the feedback circuits are more suitable a wide detection range is required. Hence, in production applications, researchers should select the construction modes most appropriate to their needs. Based on the general instruction proposed here, the heavy metal biosensors can be constructed to realize rapid, accurate and stable detection in various actual samples. At present, the stability of these microbial biosensors still need to be improved, which can be achieved by further optimization of the genetic circuits.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2019.08.006.

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