

Simple Whole-Cell Biodetection and Bioremediation of Heavy Metals Based on an Engineered Lead-Specific Operon

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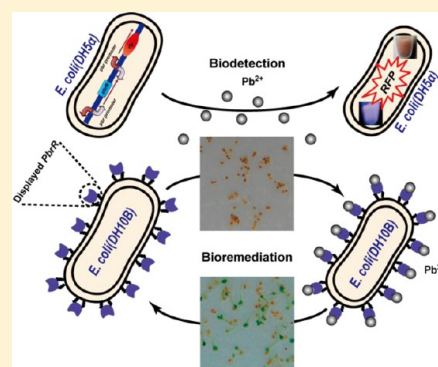
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Supporting Information

ABSTRACT: A lead-specific binding protein, PbrR, and promoter *pbr* from the lead resistance operon, *pbr*, of *Cupriavidus metallidurans* CH34 was incorporated into *E. coli* in conjunction with an engineered downstream RFP (red fluorescence protein), which allowed for highly sensitive and selective whole-cell detection of lead ions. The subsequent display of PbrR on the *E. coli* cell surface permitted selective adsorption of lead ions from solution containing various heavy metal ions. The surface-engineered *E. coli* bacteria effectively protected *Arabidopsis thaliana* seed germination from the toxicity of lead ions at high concentrations. Engineering the *E. coli* bacteria harboring these lead-specific elements from the *pbr* operon may potentially be a valuable general strategy for biodetection and bioremediation of toxic heavy metal ions in the environment.



1. INTRODUCTION

As a main group element, lead (Pb) is one of the earliest heavy metals used by humans and still plays a key role in modern industries, such as automobile manufacturing, military, electric power, metallurgy, printing, construction, and others.^{1,2} Due to the widespread use of lead in paint, pipes and plumbing materials, gasoline, batteries, ammunition, and cosmetics, lead can be found in all aspects of our environment. However, because of its nondegradable nature and physiological toxicity, lead poses a serious risk through food chain accumulation, like other heavy metals. Lead poisoning is a non-negligible risk to people's health, particularly to children, and is a public health issue on a global scale.^{3–6}

Lead pollution is present in wastewater, waste gases, or waste residues, which often contain other metals, including zinc, cadmium, and mercury.^{7,8} Simply selective detection and adsorption of lead has been a challenge. Detection techniques, such as atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectrometry (ICP-AES), are used to analyze the lead content in solution, following complicated sample preparation processes.^{9,10} Several available lead-selective chemosensors were reported for lead ion detection, but these sensors largely rely on polymer-, small molecule-, peptide-, or

nanoparticle-based sensing systems, generally rendering them impractical for use outside of a laboratory setting.^{11–16} The most current lead adsorption techniques rely on using expensive activated carbon and ion-exchange resin adsorbents that are difficult to adapt to large-scale wastewater treatment.^{17,18}

In contrast, microorganisms have evolved diverse mechanisms to maintain homeostasis and resistance to heavy metals, including lead.^{19–22} Taking advantage of a Pb^{2+} regulatory metalloprotein from *Cupriavidus metallidurans* CH34, He et al. developed a fluorescent biosensor with high selectivity and sensitivity to Pb^{2+} ions.¹² Recently, Jouanneau et al. reported the identification of four heavy metals, including lead in environmental samples using bioluminescent bacteria.²³ Moreover, we also have reported developing a gold-selective sensor for cell-based visual detection by engineering a gold-specific regulon.²⁴

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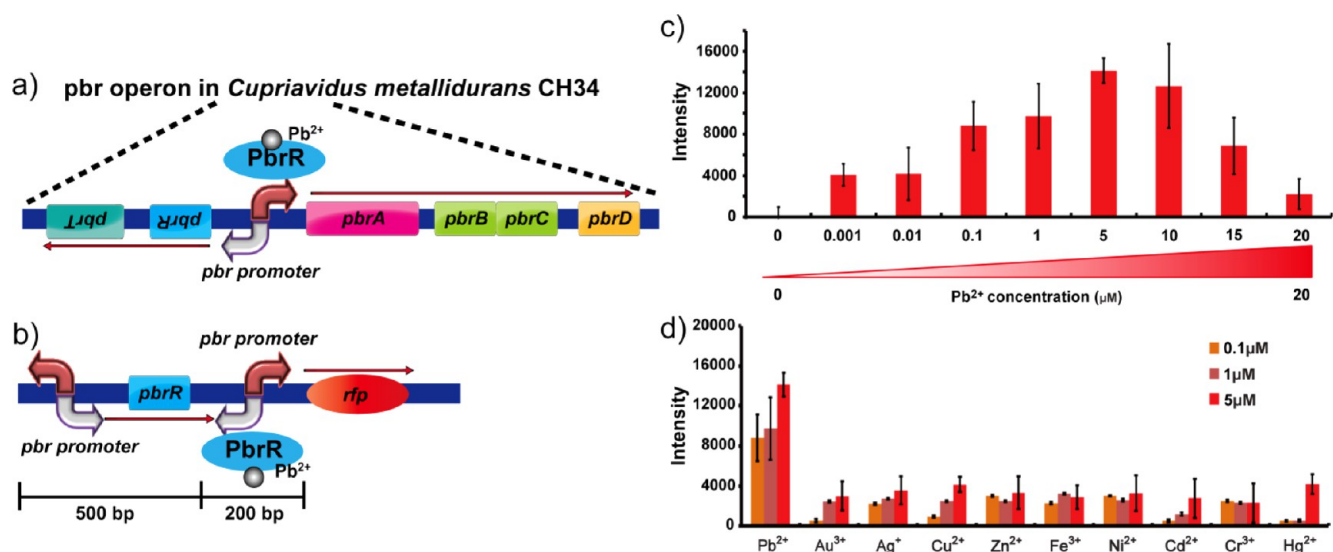


Figure 1. (a) Genetic organization of the *pbr* operon locus in the *C. metallidurans* CH34 genome. (b) Genetic organization of the lead inductive RFP expression plasmid in *E. coli*. (c) Fluorescence measurement of *E. coli* cells containing the lead induced RFP expression plasmid after gradient concentrations of Pb^{2+} induction and resuspended in PBS buffer (pH 7.4). All of the cell numbers were normalized to 3×10^7 before the tests. (d) Fluorescence measurement of *E. coli* cells containing the lead induced RFP expression plasmid with concentrations of 0.1, 1, and 5 μM each metal ions. All of the cell numbers were normalized to 3×10^7 before the tests.

Herein, we present a selective lead-sensing system and a remediation system respectively using the gene elements of the lead-specific operon *pbr*, including the lead binding protein PbrR from *C. metallidurans* CH34. The verification of engineered sensory *E. coli* cells for specific lead ion recognition was first demonstrated. The subsequent engineering of the *E. coli* cell surface to display the unique lead binding protein, PbrR, allowed the selective adsorption and immobilization of the toxic lead metal. Importantly, the cell surface-display strategy for heavy metal adsorption is especially advantageous over the traditional bioadsorption methods because of the following considerations: First, the strategy alleviates the burden of intracellular accumulation of toxic metal ions, which often results in less adsorption efficiency and poor growth of the host cells;^{24–29} Second, the strategy allows for faster interaction between the cell surface-displayed metalloproteins and metal ions in the environment; Furthermore, our previous results from the gold-specific bioadsorption system showed a higher tolerance for aqueous solutions containing toxic metal ions compared to common *E. coli* cell.²⁴ This property will be useful in increasing the survival time when treating heavy metal pollution using engineered live cells.

2. EXPERIMENTAL SECTION

2.1. Construction of Lead Biosensor. The BioBrick Vector pSB1A2 and the gene encoding RFP were constructed following a previously published protocol.³⁰ The lead selective-sensing plasmid was constructed in three steps, and all DNA fragments were amplified from *Cupriavidus metallidurans* CH34 (formerly *Ralstonia metallidurans*) genomic DNA. First, a 500-bp DNA fragment including the gene encoding PbrR and the bidirectional promoter *pbr* was amplified with PbrR-1 and PbrR-2 primers (Supporting Information, SI, Table S1). This 500-bp DNA fragment was second digested by *EcoRI* and *XbaI* and then inserted into the pSB1A2 vector following the order shown in Figure 1b. After confirmation by sequencing, the gene encoding RFP, amplified by primers RFP-1 and RFP-2, was

inserted into the vector using *XbaI* and *SpeI*. Third, a 200-bp gene fragment including only the promoter *pbr* was amplified by PbrR-3 and PbrR-4 primers, then digested by *XbaI*, and inserted into the vector between PbrR and RFP. Finally, the plasmid was transformed into *E. coli* strain DH5 α .

2.2. Lead Selectivity Detection with the *E. coli* RFP Sensor. For the lead concentration sensitivity measurements, the *E. coli* strain DH5 α containing the plasmid was cultured in LB medium until $OD_{600} = 0.6–0.8$, then induced by various concentrations (0, 0.001, 0.01, 0.1, 1, 5, 10, 15, and 20 μM) of $Pb(NO_3)_2$ (Pb^{2+}) overnight in LB medium. Meanwhile, the *E. coli* cells were also cultured in minimal medium (M9) until $OD_{600} = 0.6–0.8$, then a final concentration of 0.1, 1 and 5 μM Pb^{2+} , Au^{3+} , Ag^+ , Cu^{2+} , Zn^{2+} , Fe^{3+} , Ni^{2+} , Cd^{2+} , Cr^{3+} , or Hg^{2+} was added to the medium. All of the cells were harvested and normalized to an $OD_{600} = 1.0$ with PBS buffer (pH 7.4). For fluorescence determinations, 300 μL aliquots of each sample were applied in triplicate into a 96-well flat bottom black plates (Corning). Fluorescence was recorded using a Multi-Mode Microplate Reader (BioTek) with 558 and 583 nm filters for the excitation and emission wavelengths, respectively.

2.3. Construction of Plasmids for Surface Display on *E. coli*. The construction of OmpA expression plasmid was previously described by Wei et al.²⁴ The OmpA-PbrR fusion protein expression plasmid was constructed in two PCR steps (SI Table S1). First, the gene encoding 1–159 amino acids of OmpA and C-terminal Flag tagged-PbrR were amplified with OmpA-1/OmpA-2 and PbrR-5/PbrR-6 primers, respectively. Then, these two fragments were connected by overlap PCR using OmpA-1 and PbrR-6. After confirmation by sequencing, the PCR product was digested by *NcoI* and *XhoI* and then inserted into the pBAD vector to display the PbrR protein on the *E. coli* cell surface.

The Lpp-OmpA expression plasmid was graciously provided by Prof. Peng Chen from Peking University. The Lpp-OmpA-PbrR fusion protein expression plasmid was also constructed by two PCR steps. The Lpp-1/Lpp-2 were used to amplify the genes encoding Lpp (1–9), the signal sequence, and first nine

N-terminal amino acids of the mature *E. coli* prolipoprotein, and the outer-membrane protein OmpA (46–159), and PbrR-5/PbrR-6 primers were used to amplify the genes encoding PbrR protein, respectively. These fragments were connected using Lpp-1 and PbrR-6. The PCR product was inserted into the pBAD vector after digestion with *Nco*I and *Xho*I. As a control, the pBAD-OmpA plasmid without any displayed items was used.

2.4. SDS-PAGE and Western Blotting Analysis of Displayed Fusion Protein. The Lpp-OmpA-PbrR or OmpA-PbrR fusion proteins were expressed in *E. coli* strain DH10B. The cells were grown in LB medium containing ampicillin ($50 \mu\text{g mL}^{-1}$) with shaking overnight at 37°C . After 1:100 dilution in LB medium, the culture was grown at 37°C to an $\text{OD}_{600} = 0.6\text{--}0.8$. Protein expression was induced by the addition of arabinose to a final concentration of 0.002%, and the culture was incubated overnight at 25°C . PbrR-displayed *E. coli* cells were harvested by centrifugation (6000 rpm, 5 min) and resuspended in lysis buffer (PBS, pH 7.4). After sonication, the two fractions including the supernatant and cell membrane were separated by centrifugation (12 000 rpm, 10 min). The cell membrane fraction was treated twice with TDSET buffer (1% Triton X-100, 0.2% sodium deoxycholate, 0.1% SDS, 10 mM tetrasodium EDTA, and 10 mM Tris/HCl). After centrifugation, the precipitate was resuspended in 500 μL of PBS. Then, 100 μL of supernatant and the resuspended precipitate fractions were mixed with 10 μL of 10X loading buffer, and the samples were heated at 95°C for 10 min. After centrifugation (12 000 rpm, 10 min), the samples were loaded onto 12% SDS-PAGE gels and electrophoresed for 30 min at 75 V and then 50 min at 160 V. For Western blotting analysis, the separated proteins were transferred onto polyvinylidene difluoride membranes (Bio-Rad) at 250 mA, 4°C , and 2 h. After blocking at room temperature for 2 h in Blotto (5% nonfat dry milk in $1 \times$ TBST), the membranes were treated with 1:1000 dilutions of monoclonal anti FLAG-tag (Santa Cruz) as primary antibody overnight at 4°C followed by incubation with horseradish peroxidase (HRP)-conjugated goat antimouse secondary antibody (Santa Cruz) at room temperature for 2 h. Antibodies were detected with the ECL Western blot kit.

2.5. Bioadsorption of Lead by PbrR Displayed Cells. For lead ion adsorption, the appropriate concentration of lead ions (5 μM , 50 μM , 150 μM , or 300 μM) was added to LB medium during the induction of the Lpp-OmpA-PbrR or OmpA-PbrR fusion proteins. To measure the metal ion adsorption ability of PbrR-displayed *E. coli*, the cells were harvested from LB medium by centrifugation (4000 rpm, 10 min) and then washed with ddH_2O at least three times. The lead-adsorbed cells were lyophilized to measure the dry weight and subjected to wet ashing. Then, the samples were analyzed using the inductively coupled plasma-atomic emission spectrometer (ICP-AES; Optima 5300DV, PE, U.S.).

To measure the selectivity to adsorb lead ions, 150 μM Cu^{2+} , Cr^{3+} , Zn^{2+} , or Cd^{2+} or 0.8 μM Hg^{2+} were added during the induction to replace the lead. Additionally, a mixed solution containing zinc, cadmium, and lead ions was used to test the adsorption ability of the two engineered cells. After the treatment, the metal ion content in the samples was measured by ICP-AES.

2.6. TEM-EDX Analysis of PbrR-Displayed Cells after Lead Ion Adsorption. All transmission electron microscopy (TEM) images were recorded using a JEOL JEM-2100 electron

microscope at an accelerating bias voltage of 200 kV. After lead ion adsorption, the PbrR-displayed cells were dispersed in ddH_2O . A thick carbon film (20–30 nm) on a copper grid was dipped into the solution for one second, dried under atmospheric conditions, and then examined by TEM. Elemental analysis was performed with the energy-dispersive X-ray spectroscopy system (EDAX, AMETEK, U.S.A.) attached to the microscope.

2.7. Seed Germination of *Arabidopsis thaliana*. *Arabidopsis thaliana* seeds (wild-type) were soaked for 10 min in a 10% sodium hypochlorite solution, then rinsed and soaked for 1 h in ddH_2O . Sterilized filter papers were placed on the bottom of glass Petri dishes (diameter of 9 cm), approximately 100 seeds were distributed on glass dishes. PbrR-displayed *E. coli* cells and negative control cells (OmpA/DH10B and DH10B) were induced by the addition of arabinose to a final concentration of 0.002%. Then, the cells were harvested by centrifugation (6000 rpm, 5 min) and resuspended in ddH_2O . Lead or cadmium ions were added to the solution at final concentrations of 0, 300, or 600 mg L^{-1} . Ten mL of bacterial suspensions containing lead or cadmium ions were mixed and immediately added in the glass dishes. Metal ions solutions not containing engineered bacteria were used as the control. All of the glass Petri dishes were sealed and incubated under continuous white light at 22°C for 3 days. Seeds were considered germinated when the seed coat breakage was visible. The seed germination conditions were observed using an optical Nikon E200 microscope (Japan), and the seed germination rates were statistically analyzed.

3. RESULTS AND DISCUSSION

3.1. Measurements of the Whole Cell Lead-Specific Biosensors. The *pbr* operon is an identified MerR family transcriptional regulator that effectively differentiates Pb^{2+} from other toxic heavy metal ions, including zinc, cadmium, mercury, cobalt, silver, copper, and nickel.^{12,31,32} It is the only bacterial operon that combines the functions involved in controlling the intracellular concentration of Pb^{2+} ions by regulating the entire resistance gene. All the expressions of these genes in the operon are controlled by the PbrR protein, which responds Pb^{2+} ions induction through the bidirectional promoter *pbr*. Inspired by the recognition of the *pbr* operon (Figure 1a), we aimed to develop a system that is selectively induced by lead ions in *E. coli* with the transformed promoter *pbr*, PbrR protein, and red fluorescence protein to achieve a lead selective-sensing bacterial cells. As shown in Figure 1b, the red fluorescence protein (RFP) was regulated by the *pbr* promoter in the BioBricks vector pSB1A2, which allowed the constitutive expression of PbrR protein to respond lead ions in solution and trigger the expression of RFP when transformed in *E. coli* cells. The sensitivity of our engineered *E. coli* cells to lead was investigated. *E. coli* cells induced with graded concentrations (0, 0.001, 0.01, 0.1, 1, 5, 10, 15, and 20 μM) of $\text{Pb}(\text{NO}_3)_2$ (Pb^{2+}) were resuspended in PBS buffer (pH 7.4) and normalized to same viable count. The results demonstrated that even when the inducible concentration of lead ions reached as low as 0.001 μM (Figure 1c), the red fluorescence of the *E. coli* cells was still detected by a microplate reader, which is much more sensitive than previously reported bioluminescent bacteria.²³ Next, a final concentration of 0.1, 1 and 5 μM Pb^{2+} , Au^{3+} , Ag^+ , Cu^{2+} , Zn^{2+} , Fe^{3+} , Ni^{2+} , Cd^{2+} , Cr^{3+} , or Hg^{2+} was added into the minimal medium (M9), respectively, for metal selectivity measurements. The induced *E. coli* cells were

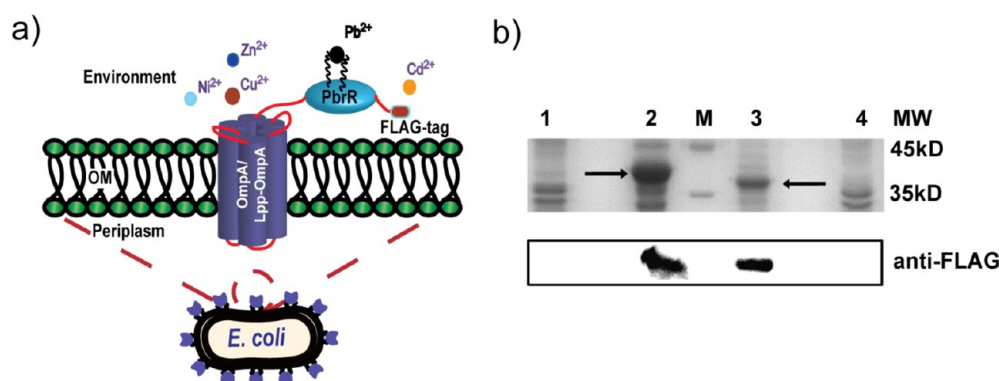


Figure 2. (a) Illustration of the lead-specific binding protein PbrR displayed on *E. coli* cell surface via the outer membrane protein OmpA or Lpp-OmpA. The blue columns represent the membrane-spanning domains of OmpA or Lpp-OmpA. PbrR is displayed on the outer surface of the *E. coli* outer membrane. (b) SDS-PAGE and immunoblotting analyses of surface-displayed PbrR protein. The black arrow on the SDS-PAGE gel indicates the OmpA-PbrR protein (lane 2) or Lpp-OmpA-PbrR protein (lane 3) expressed in bacterial membrane fraction from induced bacteria. The membrane fractions from uninduced bacteria (lane 1, 4) are used as controls. The presence of PbrR protein was further confirmed by immunoblotting analysis using anti-FLAG antibodies.

harvested by centrifugation and resuspended in PBS buffer (pH 7.4). The results showed that only in the presence of lead ions, RFP was significantly expressed and was ratiometrically detected (Figure 1d).

3.2. The Cell Surface Display of the PbrR Protein. To increase the display efficiency and minimize the potential interference with the function of proteins present on the bacterial cell surface, an appropriate lead binding protein, as a passenger candidate, and a reliable surface display system are necessary. On the basis of the unique structures, the response of fluorescent probes designed with the PbrR691, another homologous PbrR protein from *C. metallidurans* CH34, demonstrated 1000-fold selectivity for the lead ion than for other heavy metal ions.^{12,33} Thus, we sought to display PbrR protein on the *E. coli* cell surface by creating fusions with OmpA, an *E. coli* outer membrane protein.^{33,34} The Lpp-OmpA fusion system has been previously used to display proteins as large as 263 amino acids on the surface of *E. coli*, and recently, we also successfully used only OmpA for metalloprotein display.^{24,25,35} To investigate whether these two OmpA-based surface display systems more efficiently display the PbrR protein to bind lead ions, we constructed two plasmids that carry the Lpp-OmpA-PbrR or OmpA-PbrR expression genes by connecting the N-terminal of OmpA with the full-length PbrR possessing a FLAG-tag at the C-terminus (Figure 2a). These plasmids were transformed into *E. coli*, and the expression of the fusion proteins was analyzed by SDS-PAGE. As shown in Figure 2b, the expressed recombinant Lpp-OmpA-PbrR (calculated MW = 35.049 kDa) and OmpA-PbrR proteins (calculated MW = 36.788 kDa) both had molecular weights near their theoretical values on the SDS-PAGE gel, and the identities of these two fusion proteins were further verified by immunoblot analysis using anti-FLAG antibodies. As a result, the PbrR protein was expressed associated with the membrane protein fraction, and according to previous references, we inferred that the PbrR protein was probably displayed on the *E. coli* cell surface and easily accessible by metal ions from the extracellular environment.

3.3. Lead Adsorption by the Engineered *E. coli* Bacteria. Subsequently, the two PbrR-displaying *E. coli* were first incubated with 150 μM $\text{Pb}(\text{NO}_3)_2$ (Pb^{2+}) in LB medium overnight and then analyzed by ICP-AES after extensive washing. *E. coli* bacteria without induction or expressing only

OmpA were used as the controls. As shown in Figure 3a, the two *E. coli* bacteria with the surface-displayed PbrR were able to adsorb lead ions with a capacity of approximately 38.76 $\mu\text{mol g}^{-1}$ cells (OmpA-PbrR) or 55.28 $\mu\text{mol g}^{-1}$ cells (Lpp-OmpA-PbrR), which are 6- to 9-fold higher, respectively, than the undisplayed samples. Next, with various concentrations of lead ions, these two *E. coli* bacteria also adsorbed 1.13 or 1.36 $\mu\text{mol g}^{-1}$ cells even at the 5 μM concentration (Figure 3b). The display efficiency of Lpp-OmpA-PbrR appeared better than OmpA-PbrR, which is most likely due to optimization of the anchoring ability by Lpp protein.^{35,36} Interestingly, as shown in Figure 3c, the rates of effective adsorption of Lpp-OmpA-PbrR-displayed cells in the lead ions concentration of 5, 50, 150, and 300 μM ranged from 4.70% to 11.72%; however, the cells expressing OmpA-PbrR in the four concentrations ranged from 13.03% to 5.69%. The OmpA-PbrR transformed cells had a greater lead ion adsorption capacity at low concentrations compared to high concentrations; however, the adsorption capacity of Lpp-OmpA-PbrR transformed cells increased with the increasing lead ion content. We speculated that in low lead concentrations, the expression of OmpA-PbrR protein was better than Lpp-OmpA-PbrR. But the OmpA-PbrR expression may be more susceptible to high concentrations of lead ions compared to Lpp-OmpA-PbrR. As one of the most used *E. coli* surface display systems based on out-membrane protein, the Lpp-OmpA display system showed more stability on the expression of recombinant protein under lead stress. Furthermore, we also cultured the two PbrR-displayed *E. coli* bacteria in medium without lead ions and collected and resuspended the cells in lead ion water for adsorption overnight (Figure 3d). The results showed that the engineered cells had a greater lead ion adsorption capacity in water than in LB medium because the organic compounds in LB medium would compete with lead ions in solution, also indicating a potential advantage for the application of these engineered bacteria.

Next, we demonstrated that these engineered *E. coli* cells selectively adsorbed lead ions from LB medium containing heavy metal ion species separately or mixed together. The bacterial outer cell membrane is a complex chemical structure; therefore, nonspecific adsorption of other metal ions must occur to interfere with lead selective adsorption. Thus, single adsorption experiments of heavy metal ions including 150 μM Cu^{2+} , Cr^{3+} , Zn^{2+} , Cd^{2+} , or Pb^{2+} , or 0.8 μM Hg^{2+} were

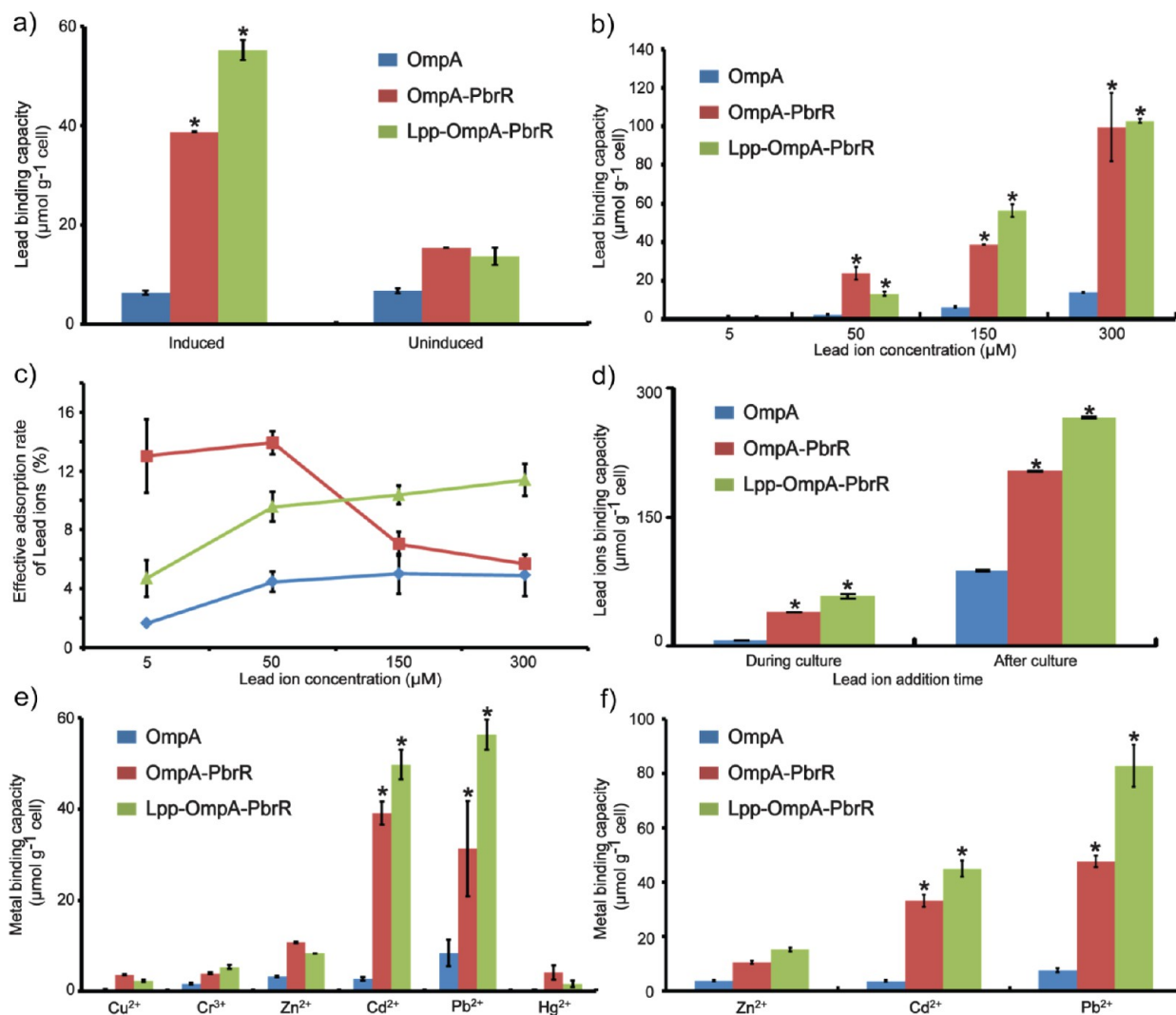


Figure 3. (a) The adsorption of lead ions by *E. coli* surface-displayed PbrR protein. The *E. coli* bacteria (DH10B strain) containing the OmpA-PbrR plasmid (red columns) or the Lpp-OmpA-PbrR plasmid (green columns) or OmpA (blue columns) were grown in LB medium containing 150 μM Pb²⁺. The lead ions were quantified by ICP-AES, and the mean values of three independently performed experiments are reported. Error bars correspond to the standard deviations. (b) Adsorption of lead ions from various concentrations of lead ions by *E. coli* surface-displayed PbrR protein. (c) The ratio of effective adsorption of PbrR protein surface-displayed *E. coli* (The *E. coli* bacteria (DH10B strain) containing the OmpA-PbrR plasmid (red line) or the Lpp-OmpA-PbrR plasmid (green line) or OmpA (blue line)) in LB medium containing various concentrations of lead ions. (d) Adsorption of lead ions by *E. coli* surface-displayed PbrR protein in lead ion solution after culture in LB medium. (e) Selective adsorption of heavy metal ions by *E. coli* surface-displayed PbrR protein. (f) Selective adsorption of heavy metal ions by *E. coli* surface-displayed PbrR protein in LB medium containing Zn²⁺, Cd²⁺, and Pb²⁺. * indicates a significant difference in metal ions binding capacity (*t* test, *P* < 0.01) between PbrR protein surface-displayed *E. coli* cells and control cells.

performed. After incubation in LB medium at 25 °C for 12 h with gentle shaking, the *E. coli* cells from each sample were collected and subjected to ICP-AES analysis. The adsorption of Pb²⁺ was significantly higher compared to the other metal ions, while the binding capacity of Cd²⁺ was only slightly lower than Pb²⁺ (Figure 3e). Considering lead water pollution often accompanied with zinc and cadmium, the adsorptions of these three heavy metal ions mixed in LB medium were tested. The results showed that the two engineered *E. coli* cells selectively bound Pb²⁺ and Cd²⁺ from the heavy metal ion mixture.

3.4. Characterization of the Lead Metal Adsorbed on PbrR-Displayed Cells. To further probe the morphology of the lead metal adsorbed on the PbrR-displayed cells, we used transmission electron microscopy and the energy-dispersive X-ray spectroscopy system (TEM-EDX) to analyze the cells after lead adsorption. The TEM image showed that the metal ions

gathered on the cell surface and formed uniform rod crystal structures approximately 100–150 nm in size (Figure 4a). Further EDX analysis on a randomly chosen area encompassing a portion of a short rod of metal crystal confirmed the element composition (Figure 4b). The results demonstrated that the lead ions were indeed adsorbed and shaped as specific crystal structures on PbrR protein-displayed cells.

3.5. Seed Germination of *Arabidopsis thaliana* under Lead and Cadmium Stress. Heavy metals such as lead, cadmium, chromium, mercury, and arsenic are toxic to plants.³⁷ Previously, scientists have developed several effective tools for immobilization and biosorption of toxic heavy metals from water and soil through complex biological engineering and transgene technology.³⁸ But it is very important that whether common bacterial cells such as engineered *E. coli* can also actually achieve this goal to a similar extent or not. *Arabidopsis*

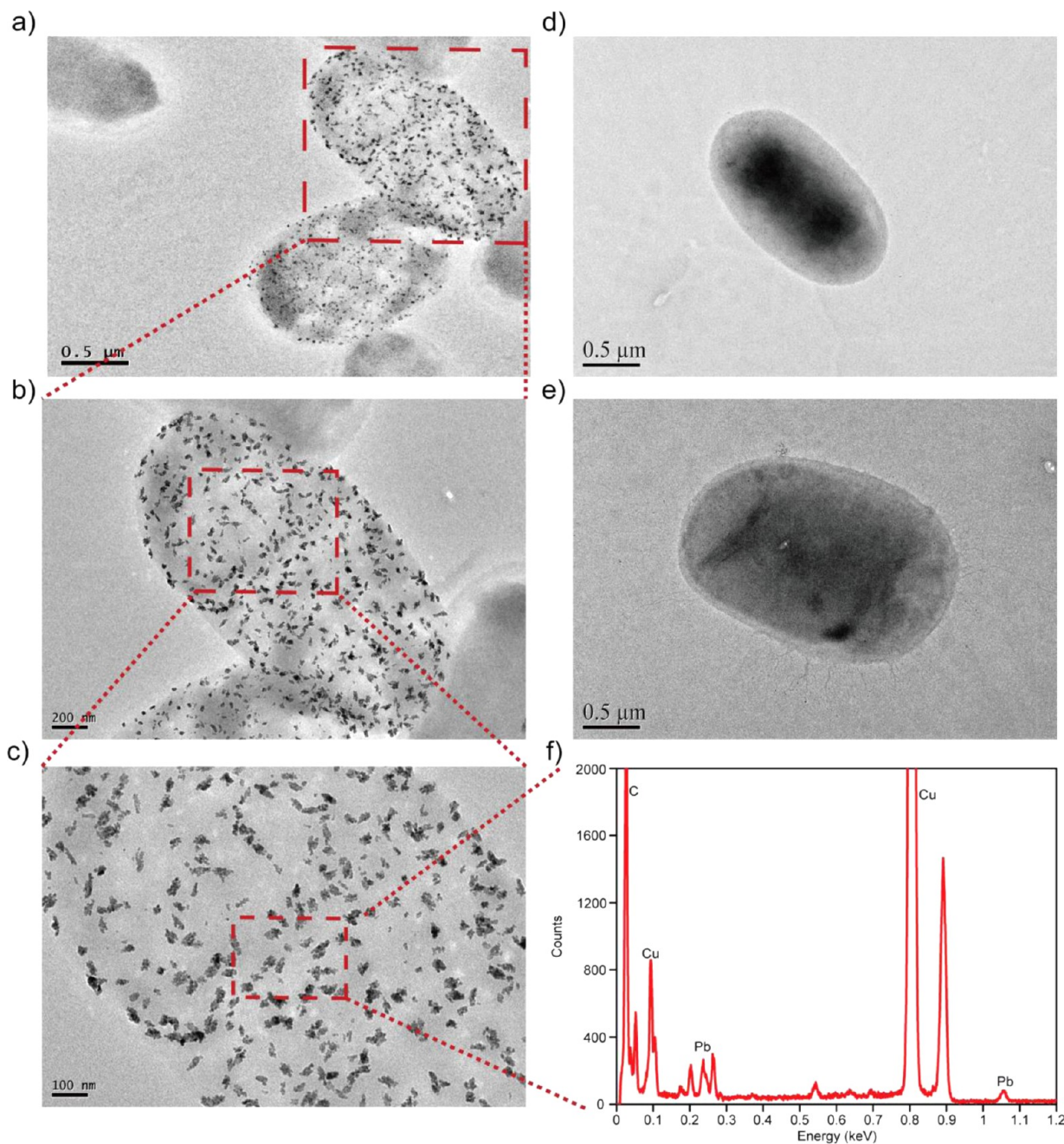


Figure 4. (a)–(c) The gradually magnified TEM images of PbrR protein surface-displayed *E. coli* cells after lead ion adsorption with the magnification from 20 000 to 100 000. (d), (e) The control TEM images of two randomly selected PbrR protein surface-displayed *E. coli* cells without lead treatment. (f) EDXA measurement of the red box from (c) representing the adsorbed lead ions.

Table 1. Percentage of Seed Germination (%) under Lead Ion Stress

	0 mg L ⁻¹ Pb ²⁺	300 mg L ⁻¹ Pb ²⁺	600 mg L ⁻¹ Pb ²⁺
control	89.30 ± 1.67	22.72 ± 2.48 ^a	8.37 ± 2.53 ^a
Lpp-OmpA-PbrR	88.75 ± 2.04	75.91 ± 3.55 ^{b,c,d}	59.15 ± 1.34 ^{b,c,d}
OmpA-PbrR	89.39 ± 2.19	76.94 ± 1.18 ^{b,c}	54.61 ± 7.27 ^{b,c}
DH10B	92.63 ± 2.06	51.27 ± 0.28 ^b	31.31 ± 2.19 ^b

^aIndicates a significant difference in the percentage of seed germination (*t* test, *P* < 0.01) between control and lead treated control samples.

^bIndicates a significant difference in the percentage of seed germination (*t* test, *P* < 0.01) between control and *E. coli* treated samples. ^cIndicates a significant difference in the percentage of seed germination (*t* test, *P* < 0.05) between PbrR surface displayed cell and *E. coli* treated samples.

^dIndicates that there is not a significant difference in the percentage of seed germination (*t* test, *P* > 0.05) between two types of PbrR surface displayed cell treated samples.

thaliana seed germination easily and intuitively demonstrates the toxicity of heavy metals to plant growth; therefore, we finally tested the bioremediation and toxicity alleviation of lead and cadmium using our PbrR-displayed bacteria. The effects of lead ions on seed germination of *Arabidopsis thaliana* seeds treated with or without engineered *E. coli* cells were observed, and the seed germination rates after treatment were also statistically analyzed, as shown in Table 1. Compared to the untreated control, the rate of seed germination significantly decreased by greater than 60% in response to 300 mg L⁻¹ lead stress and almost to only 8% after being treated with 600 mg L⁻¹ lead ions ($p < 0.01$). Gratifyingly, in the presence of our two PbrR-displayed bacteria, *Arabidopsis thaliana* seeds exposed to 300 mg L⁻¹ and 600 mg L⁻¹ lead treatment were protected from the toxicity of the lead ions and achieved a germination percentage significantly higher than the control samples without treating with PbrR-displayed bacteria. And further observation of the germinated plants has shown that the plants under 600 mg L⁻¹ lead stress still can grow as well as the control. Similarly, the effects of cadmium ions on seed germination were also observed and analyzed. As the results in Table 2 show, compared to the untreated control, the rate of

Table 2. Percentage of Seed Germination (%) under Cadmium Ion Stress

	0 mg L ⁻¹ Cd ²⁺	300 mg L ⁻¹ Cd ²⁺	600 mg L ⁻¹ Cd ²⁺
control	94.69 ± 0.90	0 ^a	0 ^a
Lpp-OmpA-PbrR	89.46 ± 2.04	44.78 ± 2.51 ^{b,c,d}	41.36 ± 7.87 ^{b,c,e}
OmpA-PbrR	83.93 ± 4.18	40.26 ± 1.86 ^{b,c}	38.17 ± 8.07 ^{b,c}
DH10B	88.23 ± 3.81	13.06 ± 2.82 ^b	4.27 ± 0.06 ^b

^aIndicates a significant difference in the percentage of seed germination (t test, $P < 0.01$) between control and cadmium treated control samples. ^bIndicates a significant difference in the percentage of seed germination (t test, $P < 0.01$) between control and *E. coli* treated samples. ^cIndicates a significant difference in the percentage of seed germination (t test, $P < 0.01$) between PbrR surface displayed cell and *E. coli* treated samples. ^dIndicates that there is not a significant difference in the percentage of seed germination (t test, $P > 0.05$) between two types of PbrR surface displayed cell treated samples. ^eIndicates a significant difference in the percentage of seed germination (t test, $P < 0.01$) between two types of PbrR surface displayed cell treated samples.

seed germination directly decreased to zero not only in response to 300 mg L⁻¹, but also to 600 mg L⁻¹ cadmium stress ($p < 0.01$). But in the presence of our two types of PbrR-displayed bacteria, nearly half of the seeds could at least germinate the as same as the cadmium-untreated seeds.

With the increasing concern regarding heavy metal pollution around the world, the technology development of selective detection and adsorption of heavy metals, including lead, has become a global challenge. The traditional methods of treating heavy metal pollution in soil and water are to use chemical chelating agents or porous polymeric materials. However, the lack of metal selectivity greatly affects the adsorption efficiency. Further, the wasted metal-complexed materials are often difficult to degrade and become secondary pollutants. Alternatively, highly selective heavy metal bioremediation methods are receiving increasing attention. Our strategy took advantage of the MerR family of proteins for their high selectivity in recognizing specific heavy metals. By engineering the specific microbial heavy metal-binding MerR protein with the cell surface display system and BioBrick system, we have developed a series of engineered bacteria for selective bioremediation and bioremediation of different heavy metals.

Specifically, by engineering a MerR family lead-specific operon and lead binding protein into *E. coli* cells, we developed a lead-sensing system on the bacterial whole cell level for the first time. This engineered *E. coli* strain enabled highly selective and sensitive recognition of Pb²⁺ ratiometrically through changes in red fluorescence. These cells are generally robust and can be regenerated with ease, which may lead to broad practical applications.

Engineering the heavy metal-specific regulators on the cell level thus provided us with a new strategy for coping with heavy metal pollution. We also have displayed the lead-binding protein, PbrR from the MerR family, on the *E. coli* cell's outer membrane surface using two OmpA-based systems. It was reported that the MerR family transporters from microorganisms responsible for Zn(zinc), Cd(cadmium), or Pb(lead) ion uptake and efflux usually could transport all three metal ions.³⁹ In addition, the lead-specific PbrR protein is from the same branch of the phylogenetic tree and shares similar binding sites with CadR, a cadmium-induced regulator of the MerR family.⁴⁰ In agreement with these previous studies, the results of metal ion adsorption measurements showed that the

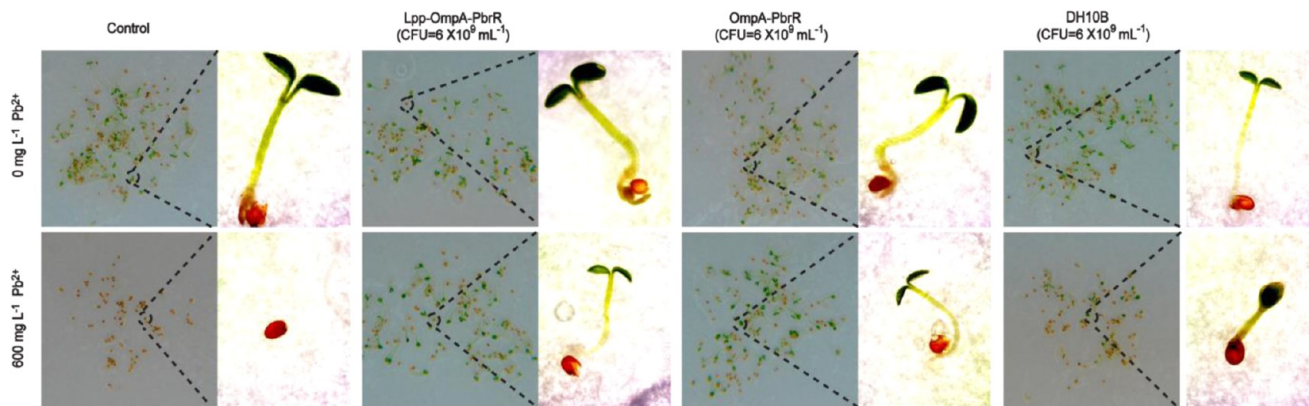


Figure 5. Observations of seed germination of *Arabidopsis thaliana* in the presence or absence of PbrR-displayed bacteria treatment with or without lead ion treatment. The black box indicated the randomly selected *Arabidopsis thaliana* seed or plant to demonstrate the germination condition, and the magnified pictures were shown on the right.

engineered *E. coli* cells could not only selectively adsorb lead ions, but also adsorb a comparable amount of cadmium ions.

The PbrR surface-displayed bacteria were examined in seed germination experiments to test the effect of improving the resistance of plants to heavy metal ions. When seed germination took place in an aqueous solution with a high concentration of lead ions, most of the lead ions were simultaneously adsorbed and immobilized by the PbrR-displayed cells, which effectively protected the seeds from the toxicity of the heavy metal ions.

Intuitively, the TEM-EDX experiment showed that the free lead ions in the aqueous solution were adsorbed on the PbrR-displayed *E. coli* cells and formed rod-like crystal structures on the cell surface. We are actively pursuing further understanding of the biomineralization process of lead crystals using our engineered bacteria, and further developments in this regard will be reported in due course. Taken together, in addition to chemical and physical treatments, engineering of the specific MerR family metal-binding protein on the surface-displayed bacterial cells provided an alternative method for the effective, renewable, and environmentally friendly bioremediation of toxic heavy metals.

■ ASSOCIATED CONTENT

● Supporting Information

Table S1 shows the PCR primers used in this work. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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