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ORIGINAL PAPER

Pigment-based whole-cell biosensor system for cadmium detection using genetically engineered *Deinococcus radiodurans*

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Abstract In this study, a colorimetric whole-cell biosensor for cadmium (Cd) was designed using a genetically engineered red pigment producing bacterium, *Deinococcus radiodurans*. Based on the previous microarray data, putative promoter regions of highly Cd-inducible genes (DR_0070, DR_0659, DR_0745, and DR_2626) were screened and used for construction of *lacZ* reporter gene cassettes. The resultant reporter cassettes were introduced into *D. radiodurans* R1 to evaluate promoter activity and specificity. Among the promoters, the one derived from DR_0659 showed the highest specificity, sensitivity, and activity in response to Cd. The Cd-inducible activity was retained in the 393-bp deletion fragment (P0659-1) of the P0569 promoter, but the expression pattern of the putative promoter fragments inferred its complex regulation. The detection range was from 10 to 1 mM of Cd. The LacZ expression was increased up to 100 μM of Cd, but sharply decreased at higher concentrations. For macroscopic detection, the sensor plasmid (pRADI-P0659-1) containing *crtI* as a reporter gene under the control of P0659-1 was introduced into a *crtI*-deleted mutant strain of *D. radiodurans* (KDH018). The color of this sensor strain (KDH081) changed from light yellow to red by the addition of Cd and had no significant response to other metals. Color change by the red pigment synthesis could be clearly recognized in a day with the naked eye and the detection range was from 50 nM to 1 mM of Cd. These results indicate that genetically engineered *D. radiodurans* (KDH081) can be used to monitor the presence of Cd macroscopically.

Keywords Cadmium · Whole-cell biosensor · Macroscopic · Red pigment · *Deinococcus radiodurans*

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Introduction

Cadmium (Cd) is a serious environmental toxicant with no known biological function. It is classified by the International Agency for Research on Cancer (IARC) as a group I carcinogen for humans and the seventh hazardous heavy metal listed by the Agency of Toxic Substances and Disease Registry [1, 2]. The worldwide recognition of toxic effects from minute concentrations of Cd has resulted in regulations to reduce its presence in the environment to very low levels. In support of this initiative, there is a clear need for reliable, efficient and cost-effective monitoring technologies for the presence of cadmium in the environment.

A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. The major advantages of using biological components in sensors are their good specificity, sensitivity, and portability. Moreover, unlike chemical or physical analyses, biological systems do not require large and expensive instruments [3]. The analysis of heavy metal ions can be carried out with biosensors using both protein-based and whole-cell-based approaches [4].

In general, microbial biosensors comprise the molecular fusion of two linked genetic elements: a sensing bioelement and a reporter gene. In most cases, the sensing element is a promoter that specifically responds to the presence or absence of the target molecule, and the reporter gene, which is fused to the sensing element, encodes a quantifiable molecule [5].

Many whole-cell microbial biosensors for detection of heavy metals, including Cd, arsenate, mercury, and copper using recombinant DNA technology, have been reported and are considered promising applications in the fields of biotechnology and environmental sciences [3, 4]. Until now, only a few whole-cell microbial biosensors for Cd detection have been developed using genetically engineered microorganisms [3, 4, 6–9]. These biosensors carry a recombinant plasmid containing *lacZ*, *luxCDABE*, or *gfp* fused with the promoter of target genes to be activated by Cd. The *β*-galactosidase (*lacZ*) and luminescence (*luxCDABE*) assays require an expensive substrate and detection device. And the fluorescence (*gfp*) bioassay also requires an elaborate fluorescence detector. However, using natural pigments as the biosensor output may overcome these issues [10].

In this study, we used a unique red pigment (deinoxanthin) producing bacterium, *Deinococcus radiodurans*, for the sensor strain, and the *crtI* gene, which is responsible for the carotenoid synthesis, as a reporter gene, to construct a simple and cost-effective whole-cell biosensor independent from the specific reagents and instruments for Cd detection. A sensor strain consisting of a *crtI*-deleted white host strain of *D. radiodurans*, in which the *crtI* gene was reintroduced downstream of Cd-inducible promoter, was constructed to detect Cd based on the conversion of the carotenoid colors.

Materials and methods

Strain, growth conditions, and medium

The bacterial strains used are listed in Table 1. *D. radiodurans* strains were grown in TGY (0.5% tryptone, 0.3% yeast extract, and 0.1% glucose) broth or on TGY plate supplemented with agar (1.5%). *Escherichia coli* strains were grown in LB (1% tryptone, 0.5% yeast extract, 0.5% NaCl) broth or on LB plate at 37 °C with aeration. Where required, antibiotics were used at the following concentrations: ampicillin (100 μg/ml) or kanamycin (50 μg/ml) for *E. coli*; kanamycin (8 μg/ml) or chloramphenicol (3 μg/ml) for *D. radiodurans*.

Enzymes and chemicals

All chemicals including heavy metals ($CdCl_2$, $CrCl_3$, $PbCl_2$, $NiCl_2$, $ZnCl_2$, $FeCl_2$, $CuCl_2$, $AsCl_3$, $MnCl_2$, and $HgCl_2$) were purchased from Sigma-Aldrich (St Louis, MO, USA). Stock solutions of each metal were prepared in distilled water. Restriction enzymes and reagents for PCR were purchased from Fermentas (Hanover, MD, USA) and Takara (Otsu, Shiga, Japan), respectively.

Construction of *β*-galactosidase (*lacZ*) and phytoene dehydrogenase (*crtI*) reporter cassettes

Chromosomal DNA was isolated from *D. radiodurans* as described by Udupa et al. [11]. The putative promoters of DR_0070 (267 bp), DR_0659 (592 bp), DR_0745 (270 bp), and DR_2626 (318 bp) were PCR amplified from the genomic DNA of *D. radiodurans* R1 using the each primer pairs (Table 2). The PCR products were digested with *Bgl*II and *Spe*I and ligated with the *Bgl*II-*Spe*I digested larger fragment of pRADZ3 to replace the putative *groESL* promoter. This procedure yielded pRADZ-P0070, pRADZ-P0659, pRADZ-P0745, and pRADZ-P2626, respectively. A set of deleted DR_0659 promoter fragments was amplified from the chromosomal DNA using each primer pairs (Table 2), digested with *Bgl*II and *Spe*I, and exchanged with the DR_0659 promoter fragment of pRADZ-P0659, generating pRADZ-P0659-1, pRADZ-P0659-2, and pRADZ-P0659-3, respectively.

To use *crtI* as a reporter gene, *crtI* was amplified from the chromosomal DNA using crtI-F/R primer pair (Table 2), digested with *Spe*I and *Xba*I, and cloned into the *lacZ* removed pRADZ3 fragment by digestion with *Spe*I and *Xba*I, generating pRADI. The promoter fragment of pRADZ-P0659-1 was obtained by digestion with *Bgl*II and *Spe*I, exchanged with the putative *groESL* promoter of pRADI, and the recombinant plasmid designated as pRADI-P0659-1.

Assay for expression of the DR_0659::*lacZ* reporter gene

A single colony of *D. radiodurans* strains was suspended in 5 ml of TGY broth and grown as a pre-culture in a shaker (200 rpm) at 30 °C for 24 h. For *β*-galactosidase activity measurements, exponentially growing recombinant *D. radiodurans* strains harboring pRADZ3 [13] derivatives were treated with metals to the indicated concentrations and time intervals. After exposure, cells (1 ml) were harvested and *β*-galactosidase activities of the pellets were measured as described by Bonacossa de Almeida et al. and Sommer et al. [14, 15].

Construction of *crtI* mutant

A fusion PCR product (3,187 bp) for *crtI* deletion was constructed in two steps. In the first step, two different

Table 1 Bacterial strains and plasmids used in this study

Designation	Relevant description	Source or references
D. radiodurans		
R1	Wild type (ATCC13939)	ATCC
KDH018	Δ*crtI*::*kan*; displacement of *crtI* with *kan* cassette from pKatAPH3; Km^r	This study
KDH041	R1 transformed with pRADZ-P0745, Cm^r	This study
KDH045	R1 transformed with pRADZ-P2626, Cm^r	This study
KDH049	R1 transformed with pRADZ-P0070, Cm^r	This study
KDH055	R1 transformed with pRADZ-P0659, Cm^r	This study
KDH084	R1 transformed with pRADZ-P0659-1, Cm^r	This study
KDH085	R1 transformed with pRADZ-P0659-2, Cm^r	This study
KDH086	R1 transformed with pRADZ-P0659-3, Cm^r	This study
KDH081	KDH018 transformed with pRADI-P0659-1, Km^r, Cm^r	This study
E. coli		
JM109	Host for plasmid subclones	Promega
Plasmids		
pGEM®-T easy	*E. coli* vector; 3 kb; Ap^r	Promega
pKatAPH3	pUC19 containing *aph* and *D. radiodurans katA* promoter; 2.3 kb; Km^r	[12]
pRADZ3	*E. coli-D. radiodurans* Shuttle Vector containing putative R1 *groESL* promoter regulated *lacZ*; 10.0 kb; Ap^r in *E. coli* and Cm^r in *D. radiodurans*	[13]
pT-Δ*crtI*	pGEM®-T easy containing *crtI*-deleted fusion PCR product; 6.2 kb; Ap^r	This study
pT-Δ*crtI::kan*	pT-Δ*crtI Sma*I::975 bp HincII fragment from pKatAPH3; 7.0 kb; Ap^r	This study
pRADZ-P0070	Exchange of *groESL* promoter with putative DR_0070 promoter in pRADZ3	This study
pRADZ-P0659	Exchange of *groESL* promoter with putative DR_0659 promoter in pRADZ3	This study
pRADZ-P0745	Exchange of *groESL* promoter with putative DR_0745 promoter in pRADZ3	This study
pRADZ-P2626	Exchange of *groESL* promoter with putative DR_2626 promoter in pRADZ3	This study
pRADZ-P0659-1	Exchange of DR_0659 promoter with DR_0659-1 promoter in pRADZ-P0659	This study
pRADZ-P0659-2	Exchange of DR_0659 promoter with DR_0659-2 promoter in pRADZ-P0659	This study
pRADZ-P0659-3	Exchange of DR_0659 promoter with DR_0659-3 promoter in pRADZ-P0659	This study
pRADI	Exchange of *lacZ* with *crtI* in pRADZ3	This study
pRADI-P0659-1	Exchange of *groESL* promoter with P0659-1 promoter in pRADI	This study

asymmetric PCRs were used to generate fragments to the left (1,629 bp) and right (1,604 bp) of *crtI* sequence using crtI-DD-UF/UR and crtI-DD-DF/DR primer pairs (Table 2), respectively. In the second step, the left and right fragments were annealed at their overlapping region and amplified by PCR as a single fragment using the outer primers (crtI-DD-UF/DR). Specifically, 1 μl of each of the two asymmetric PCR mixtures and 500 μM of each of the two outside primers were mixed together and PCR amplified. The fusion product was purified (QIAGEN, Valencia, CA, USA) and cloned into a pGEM®-T easy vector (Promega, Madison, WI, USA). The resulting plasmid was designated pT-Δ*crtI*. A 975-bp *Hinc*II fragment (*kan* cassette) from pKatAPH3 [12] was ligated to *Sma*I-digested pT-Δ*crtI* and the resulting plasmid was designated pT-Δ*crtI::kan*. A strain containing a Δ*crtI::kan* mutation was constructed by transformation of the wild-type *D. radiodurans* strain R1 [11] with PCR product (4,162 bp) amplified from plasmid pT-Δ*crtI::kan* (Table 1) using the crtI-DD-UF/DR outer primers (Table 2). Deletion of *crtI* was confirmed by the loss of red pigment and PCR using crtI-Dia-F/R primers (Table 2).

Results and discussion

Evaluation of the specificity of the putative promoters to Cd treatment

From previous microarray results [16], we selected putative promoters of the four Cd-inducible genes (DR_0070, DR_0659, DR_0745, and DR_2626) for further confirmation by β-galactosidase (*lacZ*) reporter gene assay. To examine the

Table 2 Primers used in this study

Name	Sequence (5′ to 3′)[a,b]	Description
crtI-DD-UF	CAAACTCGAGGAAGGCGAG	*crtI* deletion
crtI-DD-UR	GACTTCCGAGGTTTACTGTC**CCCGGG**GT CATACGGATTCCGCTTAA	*crtI* deletion
crtI-DD-DF	TTAAGCGGAATCCGTATGAC**CCCGGG**GA CAGTAAACCTCGGAAGTC	*crtI* deletion
crtI-DD-DR	ATGTCGGTGTCGACTTCGG	*crtI* deletion
crtI-Dia-F	GCGAGCTCGACCTCGGCAT	Diagnosis of *crtI* deletion
crtI-Dia-R	GGGGTACTTCAGCGAGGTG	Diagnosis of *crtI* deletion
DR_P0070-F	TCA**AGATCT**CTTATCGAGTGCGTGCACT	Amplification for putative promoter of DR_0070
DR_P0070-R	GTA**ACTAGT**CTTTCATGCTCCACGTTCA	Amplification for putative promoter of DR_0070
DR_P0659-F	TCA**AGATCT**GTGTTCCTTCAGAATTTC	Amplification for putative promoter of DR_0659
DR_P0659-R	GTA**ACTAGT**CAATGTCCGACCAGATGT	Amplification for putative promoter of DR_0659
DR_P0745-F	TCA**AGATCT**TGAGCATCGCGCACAGCAT	Amplification for putative promoter of DR_0745
DR_P0745-R	GTA**ACTAGT**CAGCAAAAGCAGAGGAAACA	Amplification for putative promoter of DR_0745
DR_P2626-F	TCA**AGATCT**AGGAGTGGCTGGTCTGG	Amplification for putative promoter of DR_2626
DR_P2626-R	GTA**ACTAGT**AGTAGACGCAGGAGTAGC	Amplification for putative promoter of DR_2626
crtI-F	GTG**ACTAGT*TATAGGGAAAAGGTGGTGAA CTACT***ATGACATCTGCACTTCCT	*crtI* amplification for pRADI construction
crtI-R	AAC**TCTAGA**TCAGCGCCGGATGTCCG	*crtI* amplification for pRADI construction
DR_P0659-1-F	TCA**AGATCT**GACATCAACGACTTGCAG	Forward primer for P0659-1 amplification
DR_P0659-2-F	TCA**AGATCT**AGTTGCTTCTGGGTGATG	Forward primer for P0659-3 amplification
DR_P0659-3-F	TCA**AGATCT**GAATCAACAAGACCTTGG	Forward primer for P0659-4 amplification
DR_P0659-del-R	GTA**ACTAGT**GCCTATATTCTAAATCAGTT	Reverse primer for DR_0659 promoter derivatives amplification

[a] Tags with restriction sites are in boldface characters and underlined

[b] Ribosome binding sites of *lacZ* in pRADZ3 is in boldface italic characters

specificity of promoters derived from these Cd-responsive genes, putative promoter region was fused with *lacZ* gene. The resultant reporter cassettes, pRADZ-P0070, pRADZ-P0659, pRADZ-P0745, and pRADZ-P2626, were introduced into *D. radiodurans* and analyzed for their responses to metals (Fig. 1).

The β-galactosidase activity of all the recombinant *D. radiodurans* was highly specific to Cd when compared with other metals (Fig. 1a). However, the recombinant strain KDH055 carrying pRADZ-P0659 showed the highest increase in β-galactosidase activity upon Cd exposure when compared with other recombinant strains. Next, we examined Cd dose and exposure time-dependent strength of the promoters. Although β-galactosidase activity of KDH041 carrying pRADZ-P0745 showed proportional increase to Cd concentration, KDH055 had the highest sensitivity among the recombinant strains (Fig. 1b). Expression pattern of *lacZ* upon Cd exposure time was similar to each other, except for KDH045 carrying pRADZ-P2626 (Fig. 1c). In conclusion, the putative promoter of DR_0659 was the most suitable response element for Cd biosensor development. Therefore, we analyzed the putative promoter region of DR_0659 and used it for colorimetric Cd biosensing.

Promoter analysis of DR_0659

Smaller-sized promoters are advantageous in the construction of expression cassettes with various reporter genes. Thus, we roughly identified the minimal promoter fragment required for the Cd-inducible response by simple stepwise deletion analysis of the DR_0659 promoter (Fig. 2). The 393 bp (P0659-1), 236 bp (P0659-2), and 131 bp (P0659-3) fragments of the putative DR_0659 promoter were fused with the *lacZ* gene to construct the expression vectors pRADZ-P0659-1, pRADZ-P0659-2, and pRADZ-P0659-3, respectively (Fig. 2a). Recombinant *D. radiodurans* strains, designated KDH055 and KDH084 to KDH086, were analyzed for their responses to 5 μM Cd (Fig. 2b). Although leaky LacZ expression was observed in pRADZ-P0659-3, the LacZ expression pattern of pRADZ-P0659, pRADZ-P0659-1, and pRADZ-P0659-3 in response to Cd was similar to each other. However, derepression of DR_0659 promoter activity occurred in truncated P0659-2

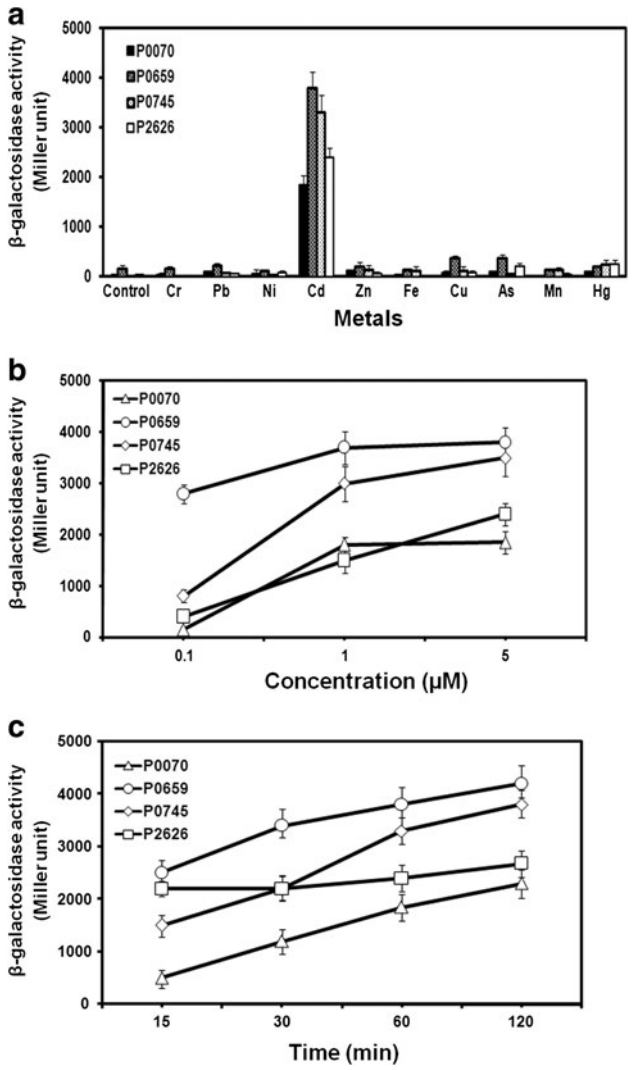

Fig. 1 Analysis of Cd-inducible activities of DR_0070, DR_0659, DR_0745, and DR_2626 promoters. **a** Response of the promoters to several metal ions. Recombinant *D. radiodurans* cells, KDH041, KDH045, KDH049, and KDH055, were grown to an OD_{600} of 1.0, and the metals were treated at 5 μM. The cells were incubated for an hour and *β*-galactosidase activity was measured. **b** Analysis of Cd concentration-dependent activities of the promoters. The exponentially growing recombinant cells were treated with 0.1, 1, or 5 μM of Cd. After Cd treatment for an hour, *β*-galactosidase activity was measured. **c** Time course analysis of the promoter activities. The exponentially growing recombinant cells were treated with 5 μM of Cd, harvested after 15, 30, 60, and 120 min of postincubation, and *β*-galactosidase activity was measured. These results are a representative of three independent experiments

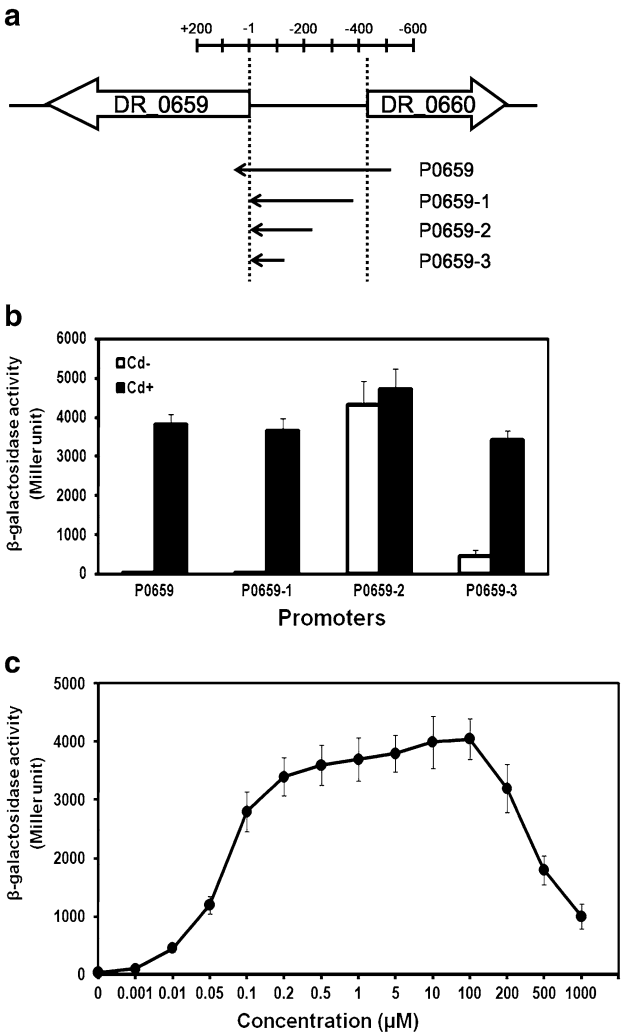

Fig. 2 Promoter analysis of the putative *D. radiodurans* DR_0659 promoter. **a** Stepwise deletion of *D. radiodurans* DR_0659 promoter. P0659, 592-bp promoter fragment; P0659-1, deleted 393-bp promoter fragment; P0659-2, deleted 236-bp promoter fragment; P0659-3, deleted 131-bp promoter fragment. **b** LacZ expression of recombinant *D. radiodurans* harboring each pRADZ-P0659 derivatives. The exponentially growing recombinant cells were treated with 5 μM of Cd, harvested after an hour of post-incubation, and *β*-galactosidase activity was measured. These results are a representative of three independent experiments. **c** Detection ranges of the P0659-1 promoter to Cd. The exponentially growing recombinant cell harboring pRADZ-P0659-1 was treated with various concentrations of Cd for an hour and *β*-galactosidase activity was measured. These results are a representative of three independent experiments

construct. One possible explanation for this behavior of pRADZ-P0659-2 and others can be provided by regulation mechanisms of metal resistance. Bacterial metal resistance systems are regulated by transcriptional factors from the MerR family [17]. It is likely that a major component of the activation mechanism, as in Mer, will involve the torsional distortion of the operator/promoter region to create an improved substrate for RNA polymerase action [18]. Some Mer members have broad specificity and have been reported to react with more than one type of metal ions; e.g., CueR reacts with Cu [I], Ag [I], and Au [I], whereas ZntR is mainly regulated by Zn [II], but also responds to Cd [II] and Pb [II] [17]. However, DR_0659 was highly specific to Cd, which indicates that it might be tightly regulated, other than Mer family. Reportedly, DNA sequences upstream of the *rrnB* P1 core promoter (−10, −35 region) increase transcription more than 300-fold

in vivo and in vitro [19]. This stimulation results from a *cis*-acting DNA sequence, the UP element, which interacts directly with the alpha subunit of RNA polymerase and from a positively acting transcription factor, FIS. Likewise, upstream region (−237 to −394) of DR_0659 might be required for its transcriptional regulation by intrinsic DNA curvature.

We further analyzed Cd detection limit of KDH084 (Fig. 2c). The LacZ expression of KDH084 was logarithmically increased up to 200 nM of Cd, and the expression was not significantly changed at the concentration from 100 nM to 100 μM of Cd. However, at higher concentrations, there was a sharp decrease in the LacZ expression that might be attributed to the toxicity of Cd. The detectable minimum Cd concentration was 10 nM. A previously reported, Cd sensor using recombinant *Bacillus subtilis* has the lowest detection limit of 3.3 nM [20], until now. However, luminescent intensity decreased sharply at higher Cd concentration due to cadmium toxicity and it also responded to lead. The lowest detection limit of Cd biosensors developed using *E. coli* and *Staphylococcus aureus* was 10 nM, but they also responded to other metals and expression of reporter gene was highly affected by Cd concentration [20, 21]. Recently, a Cd sensing system using *Pseudomonas putida* has been reported [7]. The sensor showed high sensitivity to Cd and could stably detect relatively high concentration of Cd because of inherent cadmium resistance in *P. putida*. However, this system required further steps for stable measurement and showed relatively low specificity to Cd when compared with our sensor.

Detection of Cd by red color development

Various biological systems have been used to construct whole-cell bacterial biosensors [3–5]. Generally, genetically engineered sensor bacteria contain a recombinant plasmid that carries target chemical responsive elements and/or a reporter gene(s), such as firefly (*luc*) or bacterial luciferase (*lux*), green fluorescent protein (*gfp*), and *β*-galactosidase (*lacZ*). These systems require special agent or substrates or expensive equipment for target chemical detection [22]. To overcome these drawbacks, bacterial whole-cell biosensors using natural pigment as the biosensor output have been reported. A red pigment biosynthetic gene (*crtA*) of *Rhodovulum sulfidophilum* was used for arsenite and dimethyl sulfide detection [23, 24]. A blue-green pigment synthesis of *Pseudomonas aeruginosa* was used for *N*-butyryl homoserine lactone detection [22]. A whole-cell arsenite biosensor using red pigment synthetic gene (*crtI*) of *Rhodopseudomonas palustris* was developed [25]. In this study, kanamycin resistance gene, *aph*, replaced *crtI* of *D. radiodurans* to use the red pigment (deinoxanthin) as a Cd detection marker by the naked eye. CrtI is a phytoene dehydrogenase converting colorless phytoene to reddish lycopene. Therefore, *crtI* deletion mutant (KDH018) was selected by its colony color and further confirmed by PCR (Fig. 3).

Sensor strain (KDH081) was created by transforming KDH018 with the sensor plasmid, pRADI-P0659-1. To evaluate Cd specificity of KDH081, exponentially growing cells were treated with various metals. The color of the culture treated with Cd changed to red indicating that the KDH081 responds to Cd by changing its color (Fig. 4a). However, the color of other cultures treated with metals other than Cd did not change. This result clearly indicates the Cd specificity of the sensor strain reflecting the LacZ reporter assay results.

To examine the macroscopic detection limit of KDH081, the strain was exposed to different concentrations of Cd ranging from 1 nM to 1 mM (Fig. 4b). The bacterial color change could not be recognized macroscopically at the concentrations of 1 and 10 nM. Slight color change was recognized at the concentration of 50 nM. Clear color change could be detected in

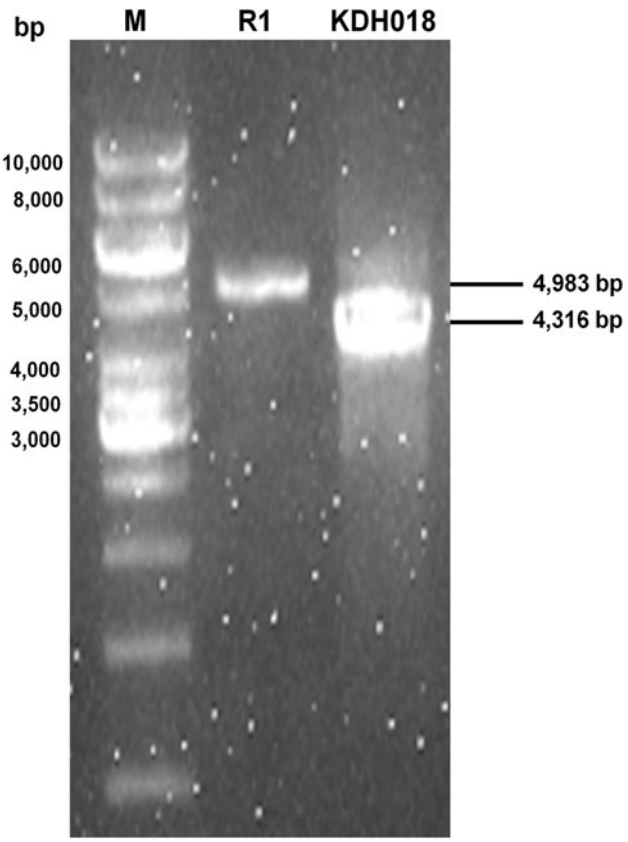

Fig. 3 Verification of *D. radiodurans crtI* gene deletion by PCR. PCRs were done with primers crtI-Dia-F and crtI-Dia-R locating outside from the mutant cassette using genomic DNA isolated from either wild-type (R1) or Δ*crtI*::*kan*^{r} mutant (KDH018) cells. The PCR product of R1 revealed a band length of 4,983 bp, whereas that of KDH018 resulted in a 4,316 bp. *M* denotes molecular standards

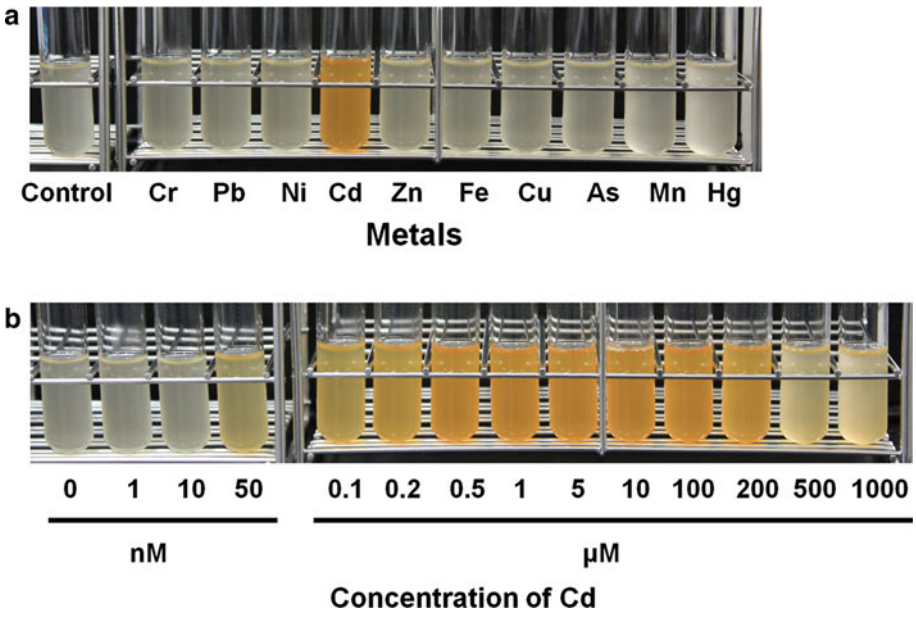

Fig. 4 Color development of the sensor strain. **a** Change of color in the Cd sensor strain in response to various metals. The exponentially growing sensor strain, KDH081, was treated with 5 μM of each metal. A photograph was taken 24 h after the addition of metals. **b** Color change of the sensor strain in response to various concentrations of Cd. The exponentially growing sensor strain was treated with various concentrations of Cd. The photograph was taken 24 h after the addition of Cd

concentrations from 100 nM to 200 μM. However, dose-dependent increase of chromaticity could not be differentiated macroscopically. Although reduction of chromaticity occurred when the sensor strain was exposed to Cd at concentrations of more than 500 μM, the highest macroscopic detectable concentration was 1 mM. When compared with results from KDH084 employing LacZ reporter assay for Cd detection, KDH081 showed relatively narrow Cd detection range (50 nM–1 mM) that might be caused from the color of the medium. However, the results clearly demonstrated that KDH081 could specifically indicate the presence of Cd by red color development enabling macroscopic monitoring of Cd in samples.

Conclusions

Very few attempts have been made to construct colorimetric whole-cell biosensors using pigment synthetic genes. To our knowledge, a colorimetric whole-cell biosensor for Cd, in which the signal is generated based on the conversion of intrinsic pigments of a host strain, has not been developed elsewhere. In this study, a pigment-based colorimetric bacterial biosensor has been successfully developed for easy and rapid macroscopic detection of Cd. A highly sensitive and specific promoter region was screened using LacZ reporter assay. Using β-galactosidase activity, Cd could be detected at nanomolar levels. The red pigment synthetic gene, *crtI*, was deleted from the host genome as an output signal. When exposed to Cd, the genetically engineered sensor strain harboring the recombinant plasmid containing the promoter fused to *crtI* changing color from light yellow to red with high specificity. The detection range of the sensor strain was from 50 nM to 1 mM. This new colorimetric whole-cell biosensing system provides a simple, stable, and cost-effective for Cd detection. Other biosensors using this pigment-based vector and host system could be constructed by inserting a promoter region that responds to a specific substance. Thus, this colorimetric system could be applied in a variety of fields.

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References

1. Fay RM, Mumtaz MM (1996) Development of a priority list of chemical mixtures occurring at 1188 hazardous waste sites, using the HazDat database. Food Chem Toxicol 34:1163–1165
2. International Agency for Research on Cancer (IARC) (1993) Beryllium, cadmium, mercury and exposure in glass manufacturing Industry. In: Monographs on the evaluation of the carcinogenic risks to humans, vol 58. IARC Scientific Publications, Lyon, pp 119–237
3. Yagi K (2007) Applications of whole-cell bacterial sensors in biotechnology and environmental science. Appl Microbiol Biotechnol 73:1251–1258
4. Verma N, Singh M (2005) Biosensors for heavy metals. Biometals 18:121–129
5. Gu MB, Mitchell RJ, Kim BC (2004) Whole-cell-based biosensors for environmental biomonitoring and application. Adv Biochem Eng Biotechnol 87:269–305
6. Shetty RS, Deo SK, Shah P, Sun Y, Rosen BP, Daunert S (2003) Luminescence-based whole-cell-sensing systems for cadmium and lead using genetically engineered bacteria. Anal Bioanal Chem 376:11–17
7. Wu CH, Le D, Mulchandani A, Chen W (2009) Optimization of a whole-cell cadmium sensor with a toggle gene circuit. Biotechnol Prog 25:898–903

8. Ivask A, Francois M, Kahru A, Dubourguier H, Virta M, Douay F (2004) Recombinant luminescent bacterial sensors for the measurement of bioavailability of cadmium and lead in soils polluted by metal smelters. Chemosphere 55:147–156
9. Park JN, Sohn MJ, Oh DB, Kwon O, Rhee SK, Hur CG, Lee SY, Gellissen G, Kang HA (2007) Identification of the cadmium-inducible *Hansenula polymorpha SEO1* gene promoter by transcriptome analysis and its application to whole-cell heavy-metal detection systems. Appl Environ Microbiol 73:5990–6000
10. Blosser RS, Gray KM (2000) Extraction of violacein from *Chromobacterium violaceum* provides a new quantitative bioassay for N-acyl homoserine lactone autoinducers. J Microbiol Methods 40:47–55
11. Udupa KS, O'Cain PA, Mattimore V, Battista JR (1994) Novel ionizing radiation-sensitive mutants of *Deinococcus radiodurans*. J Bacteriol 176:7439–7446
12. Ohba H, Satoh K, Yanagisawa T, Narumi I (2005) The radiation responsive promoter of the *Deinococcus radiodurans pprA* gene. Gene 363:133–141
13. Meima R, Lidstrom ME (2000) Characterization of the minimal replicon of a cryptic *Deinococcus radiodurans* SARK plasmid and development of versatile *Escherichia coli-D. radiodurans* shuttle vectors. J Bacteriol 66:3856–3867
14. Bonacossa de Almeida C, Coste G, Sommer S, Bailone A (2002) Quantification of RecA protein in *Deinococcus radiodurans* reveals involvement of RecA, but not LexA, in its regulation. Mol Genet Genomics 268:28–41
15. Sommer S, Bailone A, Devoret R (1985) SOS induction by thermosensitive replication mutants of miniF plasmid. Mol Gen Genet 198:456–464
16. Joe MH, Jung SW, Im SH, Lim SY, Song HP, Kwon O, Kim DH (2011) Genome-wide response of *Deinococcus radiodurans* on cadmium toxicity. J Microbiol Biotechnol 21:410–419
17. Permina EA, Kazakov AE, Kalinina OV, Gelfand MS (2006) Comparative genomics of regulation of heavy metal resistance in Eubacteria. BMC Microbiol 6:49
18. Brocklehurst KR, Megit SJ, Morby AP (2003) Characterisation of CadR from *Pseudomonas aeruginosa*: a Cd(II)-responsive MerR homologue. Biochem Biophs Res Co 308:234–239
19. Gaal T, Rao L, Estrem ST, Yang J, Wartell RM, Gourse RL (1994) Localization of the intrinsically bent DNA region upstream of the *E. coli rrnB* P1 promoter. Nucleic Acids Res 22:2344–2350
20. Tauriainen S, Karp M, Chang W, Virta M (1998) Luminescent bacterial sensor for cadmium and lead. Biosens Bioelectron 13:931–938
21. Shetty RS, Deo SK, Shah P, Sun Y, Rosen BP, Daunert S (2003) Luminescence-based whole-cell-sensing systems for cadmium and lead using genetically engineered bacteria. Anal Bioanal Chem 376:11–17
22. Yong YC, Zhong JJ (2009) A genetically engineered whole-cell pigment-based bacterial biosensing system for quantification of *N*-butyryl homoserine lactone quorum sensing signal. Biosens Bioelectron 25:41–47
23. Fujimoto H, Wakabayashi M, Yamashiro H, Maeda I, Isoda K, Kondoh M, Kawase M, Miyasaka H, Yagi K (2006) Whole-cell arsenite biosensor using photosynthetic bacterium *Rhodovulum sulfidophilum*. Appl Microbiol Biotechnol 73:332–338
24. Maeda I, Yamashiro H, Yoshioka D, Onodera M, Ueda S, Kawase M, Miyasaka H, Yagi K (2006) Colorimetric dimethyl sulfide sensor using *Rhodovulum sulfidophilum* cells based on intrinsic pigment conversion by CrtA. Appl Microbiol Biotechnol 70:397–402
25. Yoshida K, Inoue K, Takahashi Y, Ueda S, Isoda K, Yagi K, Maeda I (2008) Novel carotenoid-based biosensor for simple visual detection of arsenite: characterization and preliminary evaluation for environmental application. Appl Environ Microbiol 74:6730–6738