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Cyanobacteria carrying an *smt-lux* transcriptional fusion as biosensors for the detection of heavy metal cations

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The metal-responsive *smt* operator/promoter region of *Synechococcus* PCC7942 was fused to the *luxCDABE* genes of *Vibrio fischeri*. Plasmid DNA (pJLE23) carrying this fusion conferred metal ion-inducible luminescence to transformed cyanobacteria. *Synechococcus* PCC7942 (pJLE23) was sensitive to $ZnCl_2$ concentrations within a range of 0.5–4 μM as demonstrated by induction of luminescence. Trace levels of $CuSO_4$ and $CdCl_2$ were also detected.

Keywords: cyanobacteria; biosensor; heavy metal; lux

Introduction

Organisms carrying luminescent reporter genes fused to metal ion-inducible promoters may be utilized as biosensors for detection of bioavailable heavy metal ions in environmental samples. For example, mercury ion biosensors have been constructed by fusing the *lux* genes of *Vibrio fischeri* to the mercury resistance operon of Tn*21* [5,11]. Biosensors have also been designed for specific responses to copper, nickel, zinc, chromate, and thallium ions by fusion of the *lux* genes to a number of metal ion-responsive promoters from *Alcaligenes eutrophus* [1]. Similarly, the use of *cadA-luxAB* and *arsB-luxAB* fusions as biosensors for detection of cadmium and arsenic ions has been proposed [2]. In addition, expression of the *lux* genes under the control of the *Escherichia coli* flagellin promoter, *fliC*, is induced in the presence of a variety of metal cations including aluminum [6].

The use of gene expression driven by the *smt* operator/promoter of the cyanobacterium *Synechococcus* sp for detection of metal ions in aquatic environments has also been proposed [13]. The *smt* locus of *Synechococcus* sp consists of the prokaryotic metallothionein gene, *smtA*, and a divergently transcribed gene encoding a repressor of *smtA* transcription, designated *smtB*. Transcription of *smtA* is induced in the presence of trace metal cations including those of Cd, Zn, Cu, Hg, Co, and Ni [7]. Furthermore, fusion of the *smtA* operator/promoter region to *lacZ* confers metal cation-dependent expression of β-galactosidase in cyanobacteria [7,9]. Specifics regarding metalloregulation of the *smt* locus have been described elsewhere [4,9,13].

Cyanobacteria are photoautotrophic microbes which efficiently utilize solar energy and carbon from the air. As a result, cyanobacteria represent a low-cost, low-maintenance alternative to the use of heterotrophic microorganisms in the development of biosensors. We report here that cyanobacteria expressing the *lux* genes under the control of the *smt* operator/promoter were sensitive indicators of heavy metal cations in aqueous samples.

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Materials and methods

Cloning the smt locus

A portion of the *smt* locus from *Synechococcus* PCC7942 including *smtB*, the *smt* operator/promoter region, and the first codon of *smtA* was cloned by anchored PCR as previously described [4,7]. This DNA fragment was subsequently subcloned into pKK232–8 (Pharmacia, Piscataway, NJ, USA), creating plasmid pJLE22 (Figure 1). Plasmid pJLE23 was constructed by further subcloning an *Eco*RI-*Sal*I restriction endonuclease fragment from pJLE22 carrying this portion of the *smt* locus into plasmid pJLE11-R, a derivative of the shuttle vector pUC303 (Figure 1) [8]. pJLE11-R was previously constructed by subcloning the *luxCDABE* genes of *Vibrio fischeri* [3] as a *Sal*I cassette from plasmid pUCD320 [12] into pUC303 (unpublished data). Transcription of the *lux* cassette in pJLE23 is under the control of the *smtA* promoter.

Cyanobacterial transformation and culture conditions

Transformation of *Synechococcus* PCC7942 with pJLE23 and selection of transformants was carried out as previously described [8]. Transformants were maintained in shake flasks containing BG-11 medium at 30°C and streptomycin (25 μg ml^{-1}) with constant illumination (3000 lux) and 5% CO_2 in air. BG-11 media was prepared as described [10] and supplemented with 2 mM HEPES (pH 7.0). Cultures to be used in luminescence assays were grown in BG-11 prepared without added trace metals or EDTA.

Preparation of cultures for luminescence assays

Luminescence induction studies were performed by addition of the desired metal cation to 500 μl *Synechococcus* PCC7942 (pJLE23) ($A_{730\,nm} \approx 0.15$, cell turbidity) in polypropylene tubes. Cultures were then incubated at room temperature with illumination and 50-μl aliquots were collected for sampling at the desired time points. Experiments to detect induction by metal cations were performed in triplicate.

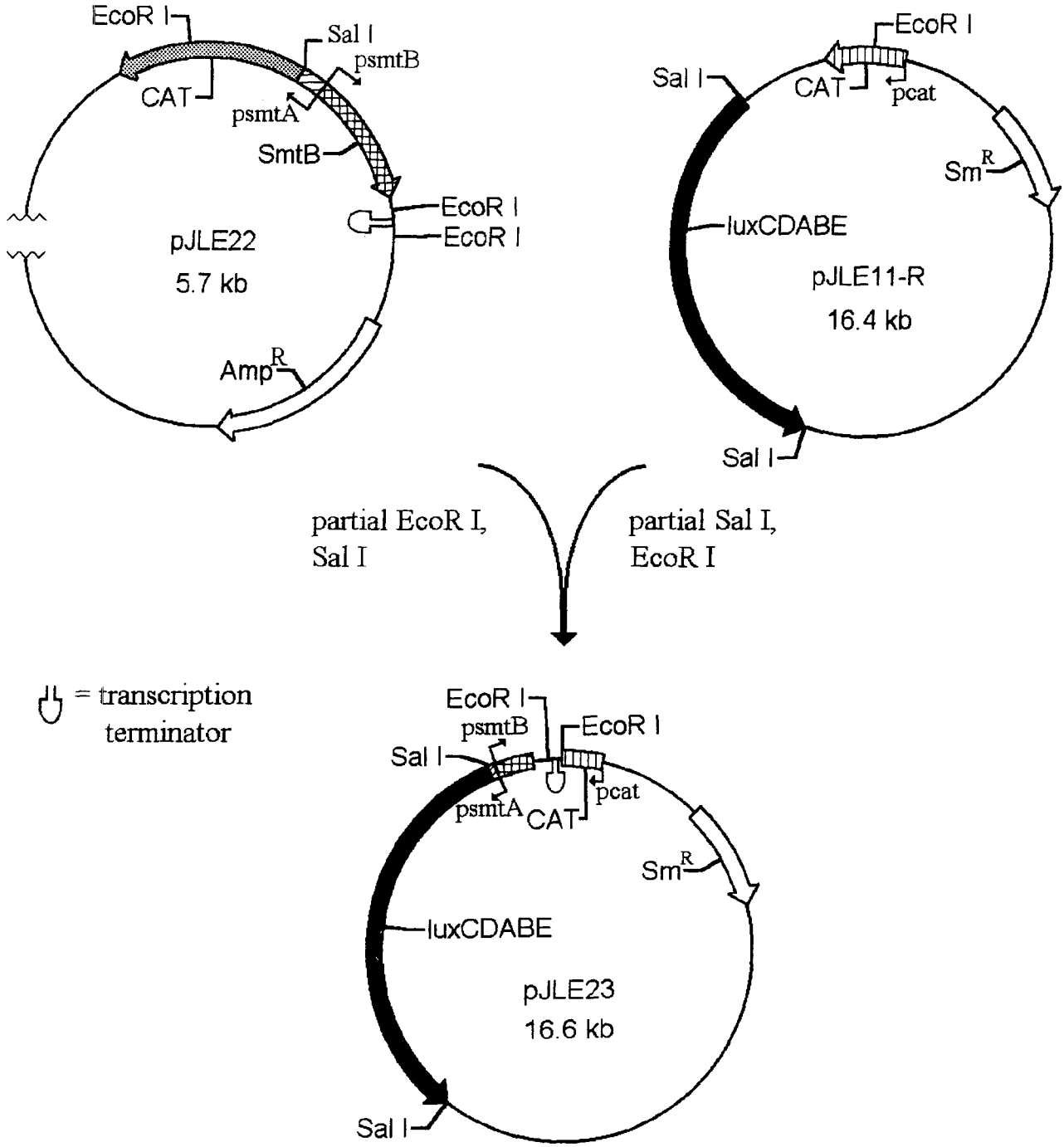

Figure 1 Construction of plasmid pJLE23. Plasmid pJLE11-R was constructed by ligation of the *luxCDABE* [12] promoterless cassette into the *SalI* site of pUC303 [8]. A portion of the *Synechococcus* PCC7942 (pJLE22) *smt* locus including *smtB*, the 100-bp operator/promoter region, and the first codon of *smtA* was subcloned into pJLE11-R to give pJLE23. Transcription of the *lux* operon in pJLE23 is under the control of the metalloregulated *smtA* promoter. Abbreviations: CAT, Amp^R and Sm^R refer to genes conferring resistance to chloramphenicol, ampicillin and streptomycin, respectively. Symbols: ⏻, transcription terminator; p, promoter.

Luminescence assay

Samples were prepared by adding 50 μl of *Synechococcus* PCC7942 (pJLE23) cells to 500 μl deionized distilled H_2O in glass tubes. The luminometer (Magic Lite Analyzer, Ciba Corning, East Walpole, MA, USA) was programmed to inject 300 μl of substrate solution (0.0125% dodecanal, 0.0125% Nonidet P40, in H_2O, Sigma, St Louis, MO, USA) into the sample immediately following the measurement of background luminescence. After a 10-s delay, light emission was measured at room temperature for 10 s and reported as counts per second (cps).

Results and discussion

In contrast to *E. coli* containing *luxCDABE*, cyanobacteria with the *lux* genes do not produce sufficient quantities of

dodecanal for maximum luminescence. In the absence of exogenous dodecanal, luminescence for cyanobacteria was less than 1% of the maximum obtainable with dodecanal in the presence or absence of metal cations (unpublished data). The construct pJLE23 contains a transcription terminator which prevented constitutive *lux* transcription (unpublished data).

To determine the sensitivity of *Synechococcus* PCC7942 (pJLE23) light emission to trace levels of heavy metal cations, cultures were incubated in the presence of various concentrations and luminescence was monitored over a 5-h time period. The luminescence profile in the presence of increasing $ZnCl_2$ concentrations is shown in Figure 2. In the absence of added metal cation, luminescence remained at a baseline level. Background luminescence was less than 0.34% of the measured maximum luminescence. Upon exposure to $ZnCl_2$, luminescence increased with time, until the 4-h time point (Figure 2). *Synechococcus* PCC7942 (pJLE23) also responded to $CuSO_4$ and to $CdCl_2$ (Figure 3). Cu^{2+} was less effective than Zn^{2+} or Cd^{2+} at a given concentration and required a longer time for induction of luminescence (Figure 3b). This may reflect differences in the intracellular cation concentration or low binding efficiency to the repressor. Metal ion concentrations which induce luminescence in this assay are comparable to the concentrations demonstrated by Huckle *et al* [7] to induce *smtA* transcription but are several fold lower than those used for detection of *lacZ* expression from the *smtA* operator/promoter.

A plot of the 60-min post-induction luminescence data points increased linearly with increasing $ZnCl_2$ from 0.5 to 2 μM (Figure 2, plot not shown). At 4 μM $ZnCl_2$, however, the relative intensity of luminescence began to decline. Exposure to higher levels of $ZnCl_2$ (8–16 μM) resulted in

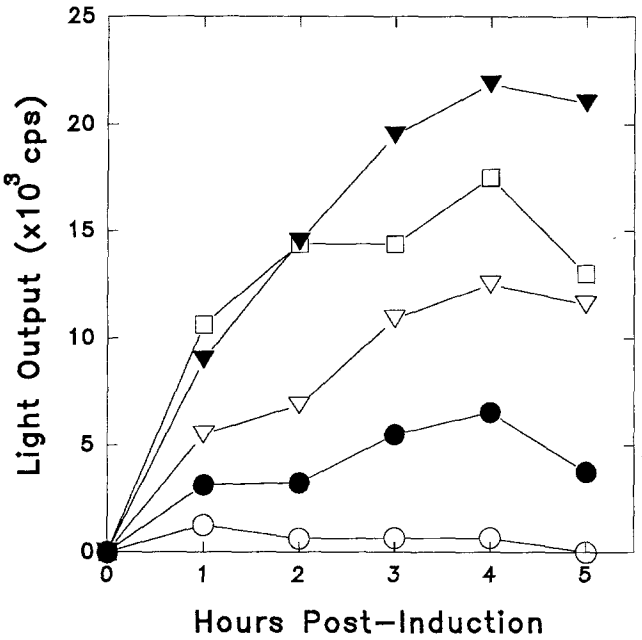

Figure 2 Induction of bioluminescence in *Synechococcus* PCC7942 (pJLE23) in response to $ZnCl_2$. (○) 0 μM $ZnCl_2$; (●) 0.5 μM $ZnCl_2$; (▽) 1 μM $ZnCl_2$; (▼) 2 μM $ZnCl_2$; (□) 4 μM $ZnCl_2$. Cells were obtained from early log phase cultures ($A_{730\,nm} \approx 0.15$, cell turbidity).

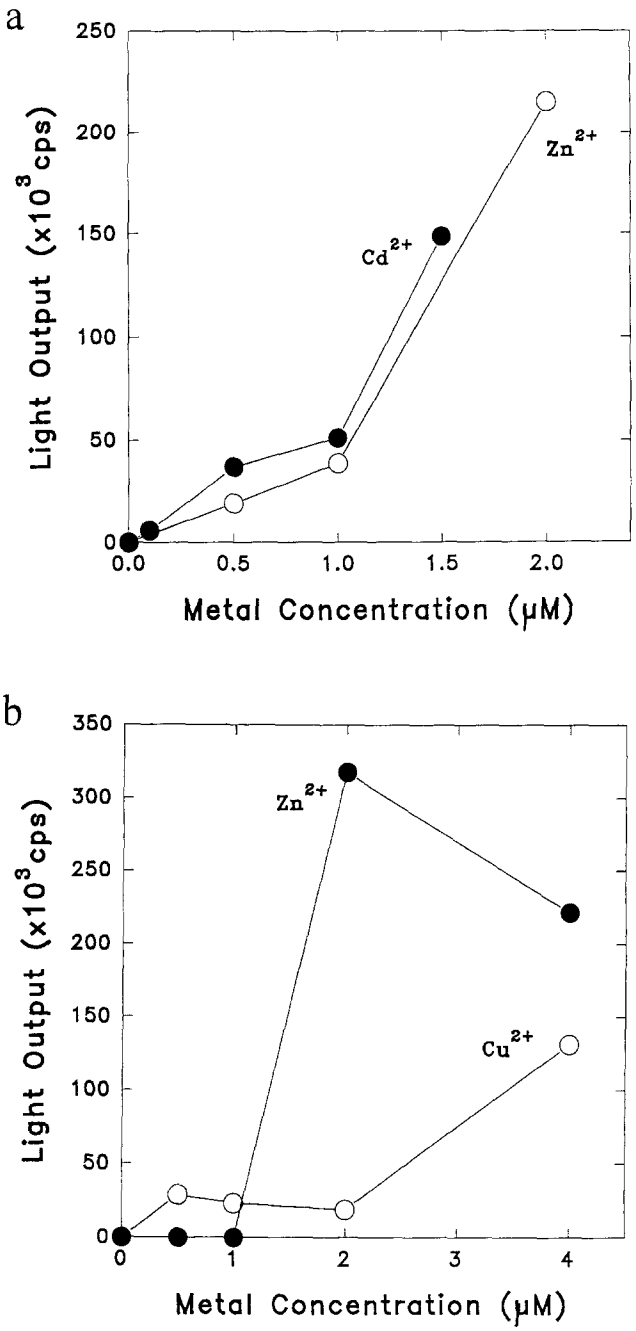

Figure 3 Induction of bioluminescence in *Synechococcus* PCC7942 (pJLE23) in response to $ZnCl_2$, $CuSO_4$ and $CdCl_2$. (a) Luminescence was measured after a 1-h exposure to $ZnCl_2$ and $CdCl_2$ and (b) after a 2-h exposure to $ZnCl_2$ and $CuSO_4$. Cells were obtained from late log phase cultures ($A_{730\,nm} \approx 0.35$, cell turbidity).

an initial increase in luminescence followed by a sharp decline toward baseline levels following the 1-h time point (data not shown). Reduced luminescence at Cu^{2+} concentrations greater than 15 μM and Cd^{2+} concentrations greater than 1.5 μM also occurred. These results are in agreement with previous data demonstrating loss of luminescence at elevated metal cation concentrations [11] and are presumably due to toxicity.

Biosensors for detection of environmental pollutants can complement traditional analytical methods by dis-

tinguishing bioavailable from unavailable forms [11]. The *smtA* operator/promoter directs metal cation-dependent expression of a promoterless *lacZ* gene and, therefore, functions as a biosensor [7]. The measurement of induction of β-galactosidase activity requires lysis of cells and a separate enzymatic determination of activity using the substrate *o*-nitrophenyl-β-D-galactopyranoside [7]. In contrast, measurement of induction in *Synechococcus* PCC7942 (pJLE23), required only the addition of dodecanal substrate to intact cells in a luminometer. The measurements were completed in 20 s. The present work demonstrates the potential for development of a simple, rapid, and economical field assay using the sensitive *smt-lux* reporter system. The results presented here indicate that *Synechococcus* PCC7942 (pJLE23) was capable of detecting trace levels of bioavailable heavy metal cations in aqueous solutions with detection of Zn^{2+} occurring at levels well below the World Health Organization recommended maximum for drinking water (5 mg L^{-1}; 80 μM). Development of a field assay instrument, collection of data on the full range of metal cations which can be detected and compounds which may interfere with metal cation detection are in progress.

Acknowledgements

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References

1 Collard JM, P Corbisier, L Diels, Q Dong, C Jeanthon, M Mergeay, S Taghavi, D van der Lelie, A Wilmotte and S Wuertz. 1994. Plasmids for heavy metal resistance in *Alcaligenes eutrophus* CH34: mechanisms and applications. FEMS Microbiol Rev 14: 405–414.
2 Corbisier P, G Ji, G Nuyts, M Mergeay and S Silver. 1993. *luxAB* gene fusions with the arsenic and cadmium resistance operons of *Staphylococcus aureus* plasmid pI258. FEMS Microbiol Lett 110: 231–238.
3 Engebrecht J, M Simon and M Silverman. 1985. Measuring gene expression with light. Science 227: 1345–1347.
4 Erbe JL, KB Taylor and LM Hall. 1995. Metalloregulation of the cyanobacterial *smt* locus: identification of *smtB* binding sites and direct interaction with metals. Nucleic Acids Res 23: 2472–2478.
5 Geiselhart L, M Osgood and DS Holmes. 1991. Construction and evaluation of a self-luminescent biosensor. Ann NY Acad Sci 646: 53–60.
6 Guzzo A, C Diorio and MS DuBow. 1991. Transcription of the *Escherichia coli fliC* gene is regulated by metal ions. Appl Environ Microbiol 57: 2255–2259.
7 Huckle JW, AP Morby, JS Turner and NJ Robinson. 1993. Isolation of a prokaryotic metallothionein locus and analysis of transcriptional control by trace metal ions. Mol Microbiol 7: 177–187.
8 Kuhlemeier CJ and GA van Arkel. 1987. Host-vector systems for gene cloning in cyanobacteria. Meth Enzymol 153: 199–215.
9 Morby AP, JS Turner, JW Huckle and NJ Robinson. 1993. SmtB is a metal-dependent repressor of the cyanobacterial metallothionein gene *smtA*: identification of a Zn inhibited DNA-protein complex. Nucleic Acids Res 21: 921–925.
10 Rippka R, J Deruelles, JB Waterbury, M Herdman and RY Stanier. 1979. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J Gen Microbiol 111: 1–61.
11 Selifonova O, R Burlage and T Barkay. 1993. Bioluminescent sensors for detection of bioavailable Hg(II) in the environment. Appl Environ Microbiol 59: 3083–3090.
12 Shaw JJ and CI Kado. 1986. Development of a *Vibrio* bioluminescence gene-set to monitor phytopathogenic bacteria during the ongoing disease process in a nondisruptive manner. Bio/Technology 4: 560–564.
13 Turner J and NJ Robinson. 1995. Cyanobacterial metallothioneins: biochemistry and molecular genetics. J Ind Microbiol 14: 119–125.