Biodegradation **8:** 105–111, 1997.

Use of a luminescent bacterial biosensor for biomonitoring and characterization of arsenic toxicity of chromated copper arsenate (CCA)

Jie Cai & Michael S. DuBow*
*Department of Microbiology and Immunology, McGill University, 3775 University Street, Montreal, Quebec, H3A 2B4, Canada (*author for correspondence)*

Accepted 15 January 1997

Key words: Escherichia coli, *ars* operon, biosensor, CCA, wood preservative, arsenic

Abstract

An arsenic oxyanion-inducible *Escherichia coli* chromosomal operon (*arsRBC*) has been previously identified. Construction of a luciferase transcriptional gene fusion (*arsB::luxAB*) showed that *ars* operon expression, plus concomitant cell luminescence, was inducible in a concentration- dependent manner by arsenic salts. The present study was conducted to evaluate the potential of the *arsB::luxAB* transcriptional gene fusion for use as a biosensor in monitoring the toxicity of arsenic compounds. Cultures from this gene fusion strain were exposed to increasing concentrations of the wood preservative chromated copper arsenate (CCA), as well as its constituents, sodium arsenate and chromated copper solution (CC). Analysis of luciferase activity revealed that the *arsB::luxAB* gene fusion was expressed in response to CCA and sodium arsenate, but not to the CC solution. The detection limit of arsenic was found to be 0.01 μg As/ml (10 parts per billion, 10 ppb) and therefore well within the range of environmental concerns. A greater induction of luminescence by arsenate was observed when cells were limited for phosphate, as phosphate can act as a competitive inhibitor of arsenate ions. Our results suggest that the *E. coli arsB::luxAB* fusion strain has a promising future as a specific and sensitive biosensor for monitoring bioavailable levels and toxicity of arsenic near sites where CCA-treated wood has been used.

Introduction

The increase of arsenic (As) concentration on the earth's surface is due to both natural sources, such as volcanic activity and weathering processes, as well as anthropogenic sources, such as mining activities, agricultural and forestry applications. One example of anthropogenic arsenic contamination in the environment is the use of arsenic-containing wood preservatives. The most extensively used wood preservative is chromated copper arsenate (CCA), which is pressurized into the wood through a process called 'Wolmanizing' (Weis & Weis 1994). Wood intended for marine uses receives 24 to 40 kg CCA per cubic meter of wood to prevent its destruction by bacteria, fungi and insects. Each of the three chemicals in CCA is known to be toxic to aquatic biota at concentrations above trace levels, and found to be leached from the treated wood in both fresh and sea water (Warner & Soloman 1990; Weis et al. 1991; Weis & Weis 1992). Chemicals leached from CCA-treated wood can affect organisms that grow on the wood itself, those that live adjacent to the CCA-treated bulkheads, and also be adsorbed onto sediments, where they can be slowly released or taken up by benthic organisms (Weis & Weis 1992, 1995; Weis et al. 1993). The rate of metal accumulation in sediments and in benthos differs with each chemical in the order: Cu > As > Cr, and decreases with distance and time (Weis and Weis 1994; Weis and Weis 1996). Benthic organisms living near CCA-treated bulkheads were found to contain elevated levels of Cu and As. The number of individuals, as well as the species diversity, were also decreased at sites adjacent to CCA-treated bulkheads (Weis & Weis 1994). Pathologic and genotoxic effects have also been observed in oysters (*Crassostrea virginica*) living on CCA-treated wood (Weis et al. 1995). In addition, CCA was shown to affect the growth of PCP-degrading bacterial species

and their ability to degrade PCP (Wall & Stratton 1994, 1995). Thus, monitoring of bioavailable amounts of CCA released by the treated wood, is important in order to detect and rectify its toxic effects. Among the three chemical constituents of CCA, arsenic is the most abundant in the environment, and known to have carcinogenic and teratogenic effects on humans upon chronic exposure (Morton & Dunnette 1994; Hartwig 1995). Therefore, the focus of this study is on biomonitoring of arsenic toxicity.

Arsenic belongs to the VA group in the periodic table. It can exist in +5, +3, 0, and −3 oxidation states in nature. The arsenic component in CCA is arsenate, the pentavalent inorganic form that is chemically similar to phosphate. The toxicity of arsenical compounds depends on their bioavailability, oxidation states, and organometalloidal forms (for a review, see Tamaki & Frankenberger 1992). For the last few decades, numerous analytical methods, including gas chromatography (GC) with flame ionization, reverse phase and high performance liquid chromatography (HPLC), mass spectrometry, X-ray fluorescence, and flame atomic absorption spectrophotometry (AAS), have been developed to detect the concentration of different forms, as well as the total amount, of arsenic in environmental samples. These methods are highly sophisticated and sensitive, but may require extensive sample pretreatment and high costs. In many cases, the samples have to be converted into other forms (e.g. arsine) in order to be detected by these analytical procedures. Moreover, the bioavailability of the original arsenic species present in a sample, and their potential toxic effects on biota, may not be reflected by these analyses. In this regard, effective means of detecting bioavailable concentrations of arsenic, and its effects on living cells, need to be fully elaborated.

Our laboratory has previously identified, using gene fusion techniques, an *Escherichia coli* chromosomal operon (*ars*), whose expression is induced in the presence of inorganic arsenic and antimony oxyanions (Diorio et al. 1995). This As/Sb-inducible operon contains three open reading frames, *arsR, B,* and *C,* which encode a *trans*-acting repressor (*arsR*) that negatively regulates the expression of the operon (Xu et al. 1996; Cai & DuBow 1996), a membrane-based pump (*arsB*), which specifically mediates extrusion of arsenite and antimonite anions, and an arsenate reductase (*arsC*). The chromosomal *ars* operon confers moderate levels of resistance to toxic forms of arsenicals and antimonials (Diorio et al. 1995; Carlin et al. 1995). The *arsR, B* and *C* genes of the chromosomal *ars* operon are highly homologous to an *E. coli* plasmid (R773)-encoded *ars* operon, which contains five ORFs in the order *arsRDABC* and encodes an arsenite/antimonite-stimulated ATP-driven efflux pump (Kaur & Rosen 1992; Wu & Rosen 1993). Further studies have been conducted to elucidate the regulation of chromosomal *ars* operon expression (Xu et al. 1996; Cai & DuBow 1996).

The aim of the present study is to examine the effects of CCA, and its constituent compounds, on bacterial cell growth and expression of the *ars* operon. Moreover, we explore the potential of an *E. coli* chromosomal *arsB::luxAB* gene fusion as a biosensor (Karube & Suzuki 1990; Danilov & Ismailov 1989) in detecting biologically important concentrations of arsenate in CCA.

Materials and methods

Bacterial strains and growth media

The bacterial strains used in this study have been described previously (Cai & DuBow 1996). *E. coli* 40 is the strain in which we isolated and sequenced the chromosomal *ars* operon (Diorio et al. 1995). *E. coli* strain LF20012 is a chromosomal *arsB::luxAB* Tet^r transcriptional fusion strain (and thus ars^-) derived from *E. coli* 40 (Cai & DuBow 1996). All assays were performed in either Luria-Bertani (LB) (Miller 1972) or LB broth reduced in phosphate, called dephosphorylated LB broth (Bukhari & Ljungquist 1977), to compare the effects of phosphate on arsenate toxicity. Tetracycline (10 μg/ml, final concentration) was routinely added to cultures of LF20012.

Chemicals

Chromated copper arsenate was purchased from Chemical Specialties Inc. (CSI, Harrisberg, NC), and is a mixture of arsenate (19% as As_2O_5, w/w), chromate (23.5% as CrO_3, w/w) and cupric oxide (9.25% as CuO, w/w). The ratio of As:Cr:Cu in this commercial formula is 13.11: 14.82: 9.11. Sodium arsenate ($Na_3AsO_4 \cdot 7H_2O$), chromium oxide (CrO_3) and cupric oxide (CuO) were purchased from American Chemicals Ltd. (A&C, Montreal, QC). A stock solution, containing identical concentrations of chromium oxide (23.5%, w/w) and cupric oxide (9.25%, w/w) as those in CCA, was prepared and designated CC solution.

All other chemicals were obtained from commercial sources.

Luciferase assays

Cultures of *E. coli* LF20012 were grown overnight in LB, or dephosphorylated LB, broth containing tetracycline in a 37 °C air shaking incubator. The overnight culture was diluted 100-fold in the same medium and growth continued until mid-log phase (A_{600}= 0.3-0.4). The cells were then exposed to increasing concentrations of sodium arsenate, CCA or CC, respectively, by adding increasing amounts of the stock solutions of each chemical to the culture, and growth was continued for 2 h. Aliquots of these cultures were removed every 30 min for luciferase activity assays. Samples were diluted in LB broth to a final A_{600} of 0.05. One milliliter (1 ml) of diluted bacterial culture (in triplicate) was placed in a 4 ml cuvette, which was then inserted into a luminometer (Tropix Optocomp I, MGM Instruments, Hamden, Connecticut). Luminescence measurements were initiated by injection of 100 µl of a dodecanal stock solution (1:100 dilution in LB, mixed by vigorous shaking). Total relative light units (RLU) were recorded for a 10 sec interval. The average RLU of each triplicate, as well as their standard deviations, were calculated by the automated program of the luminometer. Luciferase activity was expressed as the number of photons emitted per second per A_{600} unit.

Sensitivity assays

Bacterial cells were grown at 37 °C overnight in LB or dephosphorylated LB, broth containing tetracycline when required. The overnight cultures were diluted 100-fold in the same medium containing increasing concentrations of the chemicals of interest. The cultures were incubated in a shaking incubator for 6 h at 37 °C. The optical density at 600 nm of each sample (in triplicate) was recorded. The average value of the A_{600} of each culture obtained in the absence of added chemicals after 6 h incubation was used as a reference point, and set at 100%. The percent growth of each culture in the presence of added chemicals at various concentrations was calculated by dividing the average value of the observed A_{600} after 6 h incubation by that of the reference culture. The effects of the chemicals on cell growth was expressed as the percent growth versus arsenic concentration. In the case of CC, the concentration was equivalent to that used for the experiments with CCA. The LD_{50} values of each sample were defined as the elemental concentrations of arsenic in each chemical (except in CC, as designated above) that caused a 50% reduction in cell growth, and obtained according to the above growth curves.

Results

Expression of the arsB::luxAB *gene fusion is specific for arsenic in CCA*

To evaluate the effects of CCA on the expression of the *ars* operon, and the potential of the *arsB::luxAB* gene fusion for use as a biosensor in detecting bioavailable levels of arsenic compounds, bacterial cells containing this gene fusion (LF20012) were exposed to sublethal levels of CCA. Luciferase activity was analyzed as described in Materials and methods. Two other compounds, sodium arsenate, which is the form of arsenic in CCA, and the CC solution, which contains identical concentrations of chromate and cupric oxide as in CCA, but lacks arsenic, were also examined. The results showed that the *arsB::luxAB* gene fusion can be induced by CCA in both LB (Figure 1D) and dephosphorylated LB broth (Figure 1A), but not by CC (Figure 1C and F), indicating that expression of the *arsB::luxAB* gene fusion is specific for the arsenic oxyanion of CCA. These results were supported by the inducible expression of cell luminescence by sodium arsenate (Figure 1B and E). Expression of luciferase is concentration-dependent within the sublethal concentrations tested, with an assay time of 60 min or less, and can be detected at arsenic concentration as low as 0.01 µg As/ml (10 parts per billion, 10 ppb). Optimum levels of luciferase activity were achieved at 60 min post-exposure to 0.1 to 1.0 µg/ml arsenic in CCA or sodium arsenate, with a maximum induction of 50- to 90-fold, depending on the experimental conditions used (Figure 1A, B, D, and E). A decrease in the level of luminescence was observed after 60 min when cells were exposed to CCA in dephosphorylated LB but not in LB (see Discussion). Arsenic concentrations greater than 1.0 µg As/ml caused a reduction of luminescence (data not shown) presumably due to toxic effects of arsenic on cell physiology and luciferase cofactor levels (Meighen 1991; Guzzo et al. 1992).

Effects of CCA on bacterial growth

The effect of CCA on bacterial growth was evaluated as described in Materials and methods. The *arsB::luxAB*

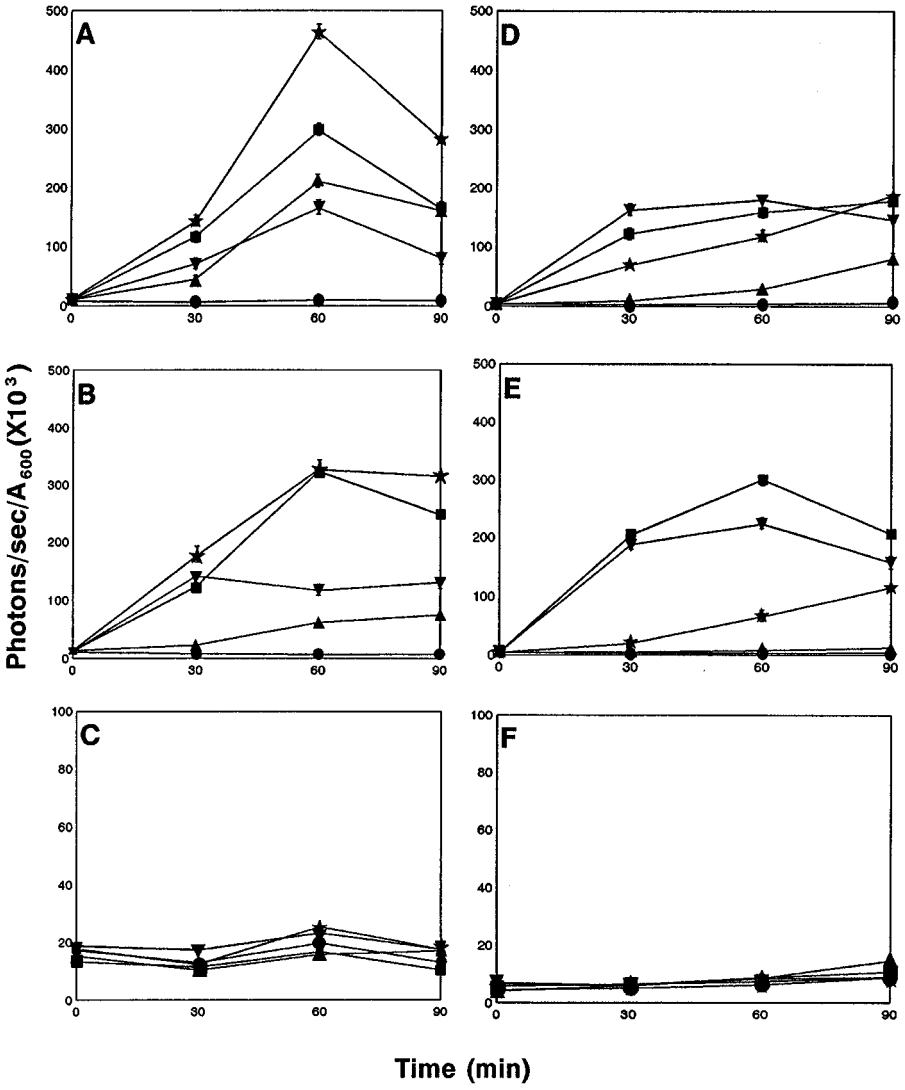

Figure 1. Luminescence of the *arsB::luxAB* fusion strain LF20012 in the presence of CCA, arsenate, and CC. Cells growing at mid-log phase in dephosphorylated LB (A–C) or regular LB (D–F) were exposed to increasing concentrations of arsenic in CCA (A and D), sodium arsenate (B and E), as well as CC solution (C and F), whose concentration was equivalent to that of CCA. Samples were removed every 30 min. Luciferase activity was measured (in triplicate) as described in Materials and methods. The standard deviations are shown by error bars. ●, 0 μg/ml; ▲, 0.01 μg/ml; ⋆, 0.1 μg/ml; ■, 0.5 μg/ml; ▼, 1.0 μg/ml.

fusion strain (*ars*$^{-}$) was found to be more sensitive to arsenic than the wild type strain (Figure 2A, B and E), consistent with previous studies of *E. coli* strains in which the *ars* operon is no longer functional (Diorio et al. 1995; Carlin et al. 1995). The toxicity of CCA or sodium arsenate was higher in dephosphorylated LB than in LB medium, as revealed by the LD_{50} values shown in Table 1. However, when cultures were exposed to CCA in LB broth, there was no significant difference in LD_{50} values between the *arsB::luxAB* fusion strain and the wild type strain at the concentrations tested (Figure 2D and Table 1). This result may be due to the predominant toxic effects of chromium and copper, over those of arsenic, when phosphate is abundant, and is supported by the observation that wild type cells were more sensitive to CC than to arsenate (Table 1). Nonetheless, greater sensitivity of both strains was observed in media containing CCA than those containing CC only, suggesting a synergistic toxic effect of the three constituents of CCA on the cells.

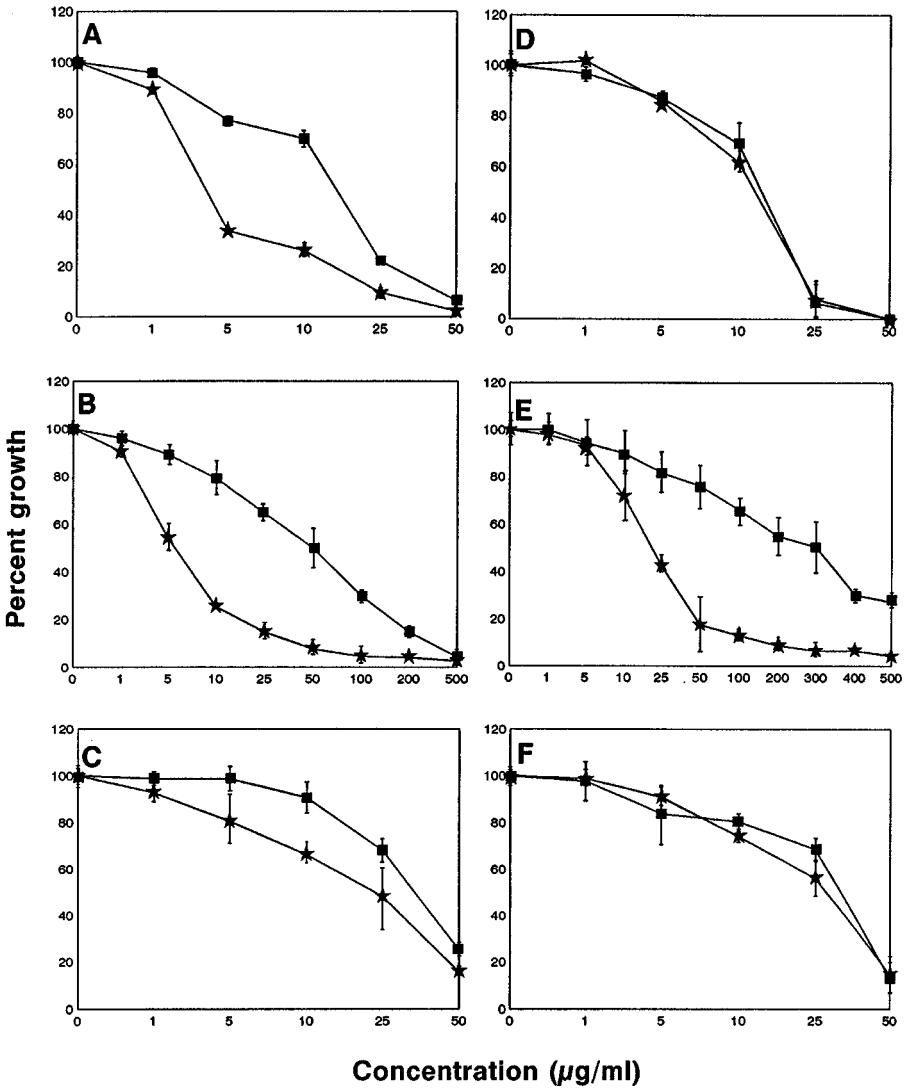

Figure 2. Growth inhibition in the presence of CCA, arsenate and CC. Cells were grown at 37 °C in a shaking air incubator in the presence of increasing concentrations of CCA (A and D), sodium arsenate (B and E), and CC (C and F) in either dephosphorylated LB (A–C) or regular LB (D–F). After 6 h, the absorbance at 600 nm (A_{600}) of each sample (in triplicate) was recorded. The average percent of growth (compared with no added chemical) of each sample was calculated as described in Materials and methods, and plotted on the Y axes. The standard deviations are shown by error bars. The concentrations indicated on the X axes are the elemental concentrations of arsenic (μg/ml), except in the case of CC, whose concentration is equivalent to that used for the experiments with CCA. ⋆, LF20012 (*ars*$^-$); ■, 40 (*ars*$^+$).

Table 1. LD_{50}(μg As/ml)[a] of chemicals to *E. coli* 40 (*ars*$^+$) and *E. coli* LF20012 (*ars*$^-$)

Chemical	CCA		Na arsenate		CC[b]	
culture medium	de-P LB[c]	LB	de-P LB	LB	de-P LB	LB
E. coli strain						
40 (*ars*$^+$)	17	14.8	50	300	30	35
LF20012(*ars*$^-$)	3.9	13.4	5.8	20.8	23.5	30

[a] Calculated as described in Materials and methods.
[b] The concentrations of chromate and cupric oxide in CC are equivalent to those in CCA.
[c] Dephosphorylated LB.

Discussion

In the present study, an *E. coli arsB::luxAB* luciferase gene fusion strain (LF20012) was shown to increase luminescence in the presence of increasing concentrations of the commonly used, arsenic-containing wood preservative, CCA, as well as to sodium arsenate, but not to the other two components (chromate and cupric oxide) of CCA. It is interesting to note that the *arsB::luxAB* gene fusion is expressed, in general, to a higher level in dephosphorylated LB medium than in LB medium when the same amount of arsenic was present in the media (Figure 1). These results may be due, in part, to the fact that arsenate is taken up by most organisms through phosphate transport systems. In *E. coli*, for example, there are two major inorganic phosphate transport systems, the low affinity Pit system and the high affinity Pst system (Willsky and Malamy 1980). The Pit system has equal affinity for both phosphate and arsenate, while the Pst system has a higher affinity for phosphate than for arsenate (Willsky and Malamy 1980). Therefore, the depletion of phosphate (in dephosphorylated LB broth) may favor increased arsenate uptake by the cells through both Pit and Pst systems due to the decreased phosphate competition for arsenate uptake, leading to higher levels of *arsB::luxAB* gene fusion expression in response to arsenate. On the other hand, a general decrease in the level of luminescence was observed after 60 min of cellular exposure to CCA or arsenate. In particular, a greater decrease was seen after 60 min in dephosphorylated LB with CCA than in LB medium. This phenomenon could be attributed to several factors. First, cells begin to enter stationary phase at the time between 60 to 90 min under these conditions, with concomitantly slower metabolic rates and reduced oxygen uptake, production of luciferase enzyme and its substrate ($FMNH_2$), all of which can lead to a decrease in luminescence in general (see Cai & DuBow 1996, for example). Secondly, Blouin et al. (1996) suggest that formation of the luciferase-FMNHOOH complex is a primary determining factor for bioluminescence, whereas regeneration of $FMNH_2$, the substrate for the luciferase enzyme, depends on the level of NAD(P)H+H^+ and NADH-FMN oxidoreductase (Blouin et al. 1996). Arsenate is known to uncouple oxidative phosphorylation by arsenolysis [29, 30], which may affect the intracellular level of NAD(P)H+H^+ generated via the electron transport system, thus decreasing cellular levels of $FMNH_2$. Since there is presumably a higher level of intracellular arsenate in phosphate depleted LB medium than in regular LB medium, this toxic effect may be more pronounced in dephosphorylated LB medium with time, leading to a more rapid decrease in luminescence in dephosphorylated LB.

The advantage of this bacterial gene fusion as an arsenic biosensor lies in its specificity, sensitivity, selectivity, and simplicity of operation. Our results have shown that this biosensor is specific for arsenic, and the detection limit can be as low as 0.01 μg/ml, which is within the federal drinking water standard (i.e. 0.05 μg As/ml). Secondly, bacterial cells are easy to maintain. It has been recently reported that a recombinant *E. coli* biosensor for organophosphorus neurotoxins can be stably maintained at 4 °C for over 2 months (Rainina et al. 1996). Our *arsB*::*luxAB* gene fusion strain has been found to be stable for at least 6 months when stored in 25% (v/v) glycerol at −20 °C (data not shown). Moreover, bacterial cells can be easily and inexpensively propagated, luminescence assays are simple to perform, and results can be rapidly obtained after sampling. It is interesting to note that a non-linear dependence of luminescence versus the concentration of arsenic tested was observed in our system, suggesting a complex intracellular biochemistry of arsenic, as supported by our previous observation that even an *ars*$^-$ mutant can grow in arsenite or arsenate concentrations above those that induce *ars* operon expression (Diorio et al. 1995), suggesting that the *ars* operon is not the only system acting on toxic arsenic oxyanions. Nonetheless, the value of this gene fusion strain as a living bacterial biosensor cannot be underestimated. With proper control analyses, the use of living biosensor systems can enhance analytical chemistry in quantitatively evaluating not only the levels, but also the bioavailability and toxic effects, of arsenic in the environmental samples. Thus, the use of these luminescent gene fusion biosensors in detecting environmental arsenic contamination may hold promise in environmental assessment.

Acknowledgments

We thank C. Diorio for technical assistance and helpful discussions during the course of this work, the reviewers for their comments and suggestions, and F. Sieder for computer aid in preparing the manuscript. This work was supported by a grant (97043) from the Centre for the Alternatives to Animal Testing, USA.

References

Blouin K, Walker SG, Smit J & Turner RFB (1996) Characterization of *in vivo* reporter systems for gene expression and biosensor applications based on *luxAB* luciferase genes. Appl. Environ. Microbiol. 62: 2013–2021

Bukhari AI & Ljungquist E (1977) Bacteriophage Mu: Methods for cultivation and use. In: Bukhari AI, Shapiro JA & Adhya SL (Eds) DNA Insertion Elements, Plasmids, and Episomes (pp 751–756). Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Cai J & DuBow MS (1996) Expression of the *Escherichia coli* chromosomal *ars* operon. Can. J. Microbiol. 42: 662–671

Carlin A, Shi W, Dey S & Rosen BP (1995) The *ars* operon of *Escherichia coli* confers arsenical and antimonial resistance. J. Bacteriol. 177: 981–986

Danilov VS & Ismailov AD (1989) Bacterial luciferase as a biosensor of biologically active compounds. In: Wise DL (Ed) Applied Biosensors. Biotechnology series. Davies JE (Series ed) (pp 39–78). Butterworth Publishers. Stoneham, MA

Diorio C, Cai J, Marmor JS, Shinder R & DuBow MS (1995) An *Escherichia coli ars* operon homolog is functional in arsenic detoxification & is conserved in Gram-negative bacteria. J. Bacteriol. 177: 2050–2056

Guzzo J, Guzzo A & DuBow MS (1992) Characterization of the effects of aluminum on luciferase biosensors for the detection of ecotoxicity. Toxicol. Lett. 64/65: 687–693

Hartwig A (1995) Current aspects in metal genotoxicity. Biometals 8: 3–11

Karube I & Suzuki M (1990) Microbial biosensors (Chapter 6). In: Cass AEG (Ed) Biosensors, a Practical Approach. The Practical Approach Series. Rickwood D & Hames BD (Series Eds) (pp 155–169). IRL Press at Oxford University Press, NY

Kaur P & Rosen BP (1992) Plasmid-encoded resistance to arsenic and antimony. Plasmid 27: 29–40

Meighen EA (1991) Molecular biology of bacterial bioluminescence. Microbiol. Rev. 55: 123–142

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory Cold Spring Harbor. NY

Morton WE & Dunnette DA (1994) Health effects of environmental arsenic. In: Nriagu JO (Ed) Arsenic in the Environment. Part II: Human Health and Ecosystem Effects (pp 17–34). John Wiley and Sons Inc, NY

Rainina EI, Efremenco EN, Varfolomeyev SD, Simonian AL & Wild JR (1996) The development of a new biosensor based on recombinant *E. coli* for the direct detection of organophosphorus neurotoxins. Biosensors and Bioelectronics 11: 991–1000

Summers AO & Silver S (1978) Microbial transformations of metals. Ann. Rev. Microbiol. 32: 637–672

Tamaki S & Frankenberger Jr WT (1992) Environmental biochemistry of arsenic. Rev. Environ. Contam. Toxicol. 124: 79–110

Valee BL, Ulmer DD & Wacker WEC (1960) Arsenic toxicology and biochemistry. Arch. Ind. Health 21: 132–151

Wall AJ & Stratton GW (1995) Effects of chromated-copper-arsenate wood preservative on the growth of a pentachlorophenol degrading bacterium. Water, Air & Soil Pollution 82: 723–737

Wall AJ & Stratton GW (1994) Effects of a chromated-copper-arsenate wood preservative on the bacterial degradation of pentachlorophenol. Can. J. Microbiol. 40: 388–392

Warner JE & Solomon KR (1990) Acidity as a factor in leaching of copper, chromium, and arsenic from CCA-treated dimension lumber. Environ. Toxicol. Chem. 9: 1331–1337

Willsky GR & Malamy MH (1980) Characterization of two genetically separable inorganic phosphate transport systems in *Escherichia coli*. J. Bacteriol. 144: 356–365

Willsky GR & Malamy MH (1980) Effect of arsenate on inorganic phosphate transport in *Escherichia coli*. J. Bacteriol. 144: 366–374

Weis JS & Weis P (1994) Effects of contaminants from chromated copper arsenate-treated lumber on benthos. Arch. Environ. Contam. Toxicol. 26: 103–109

Weis P, Weis JS & Coohill L (1991) Toxicity to estuarine organisms of leachates from chromated copper arsenate treated wood. Arch. Environ. Contam. Toxicol. 20: 188–194

Weis P & Weis JS (1992) Toxicity of construction materials in the marine environment: A comparison of chromated copper arsenate treated wood and recycled plastic. Arch. Environ. Contam. Toxicol. 22: 99–106

Weis JS & Weis P (1995) Effects of chromated copper arsenate (CCA) pressure-treated wood in the aquatic environment. Ambio, 24: 269–274

Weis P, Weis JS & Procter T (1993) Copper, chromium and arsenic in sediments adjacent to wood treated with chromated-copper-arsenate. Estuar. Coast Shelf Sci. 36: 71–79

Weis JS & Weis P (1996) Reduction in toxicity of chromated copper arsenate (CCA)-treated wood as assessed by community study. Mar. Environ. Res. 41: 15–25

Weis P, Weis JS, Couch J, Daniels C & Chen T (1995) Pathological and genotoxicological observations in oysters (*Crassostrea virginica*) living on chromated copper arsenate (CCA)-treated wood. Mar. Environ. Res. 39: 275–278

Wu J & Rosen BP (1993) The *arsD* gene encodes a second *trans*-acting regulatory protein of the plasmid encoded arsenic resistance operon. Mol. Microbiol. 8: 615–623

Xu C, Shi W & Rosen BP (1996) The chromosomal *arsR* gene of *Escherichia coli* encodes a *trans*-acting metalloregulatory protein. J. Biol. Chem. 271: 2427–2432