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Recombinant Luminescent Bacteria for Measuring Bioavailable Arsenite and Antimonite

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Luminescent bacterial strains for the measurement of bioavailable arsenite and antimony were constructed. The expression of firefly luciferase was controlled by the regulatory unit of the *ars* operon of *Staphylococcus aureus* plasmid pI258 in recombinant plasmid pTOO21, with *S. aureus* RN4220, *Bacillus subtilis* BR151, and *Escherichia coli* MC1061 as host strains. Strain RN4220(pTOO21) was found to be the most sensitive for metal detection responding to arsenite, antimonite, and cadmium, the lowest detectable concentrations being 100, 33, and 330 nM, respectively. Strains BR151(pTOO21) and MC1061(pTOO21) responded to arsenite, arsenate, antimonite, and cadmium, the lowest detectable concentrations being 3.3 and 330 μM and 330 and 330 nM with BR151(pTOO21), respectively, and 3.3, 33, 3.3, and 33 μM with MC1061(pTOO21), respectively. In the absence of the mentioned ions, the expression of luciferase was repressed and only a small amount of background light was emitted. Other ions did not notably interfere with the measurement in any of the strains tested. Freeze-drying of the cells did not decrease the sensitivity of the detection of arsenite; however, the induction coefficients were somewhat lower.

The measurement of metal bioavailability by traditional analytical methods is difficult. However, the bioavailability of metals is important in the determination of metal toxicity (8). Genetically modified microbes are regarded as a promising means of assessing the bioavailability of environmental substances (14).

Numerous nonspecific microbial whole-cell sensors have been developed which react to nearly any kind of toxic substance (14). A novel approach for a microbial whole-cell sensor is to use recombinant-DNA technology to construct a plasmid or other vector system in which a strictly regulated promoter is connected to a sensitive reporter gene. The most interesting promoters for environmental analysis are found in bacteria which survive in extreme environments contaminated by, for example, heavy metals or organic compounds. The ability of bacteria to survive in a contaminated environment is usually based on a genetically encoded resistance system, the expression of which is precisely regulated (33). Sensor bacteria in which this promoter-reporter gene concept is operable have been developed to detect mercury (28, 34, 37), arsenic ions (5, 12, 27), and xenobiotic compounds (10, 15), among other things.

Luciferase genes are widely used reporter genes in prokaryotic as well as eukaryotic systems, because they provide the sensitive and simple detection of gene expression and regulation (38). The quantification of light emission, i.e., bioluminescence, is one of the most sensitive means of detection, and it can be measured with a liquid scintillation counter or a luminometer or even with X-ray film. The most commonly used luciferases are the firefly luciferase (*Photinus pyralis*) and the bacterial luciferases of *Vibrio harveyi* and *Vibrio fischeri*. These luciferases from different groups have no apparent evolutionary relationships; even the reactions they catalyze are different. The bacterial luciferases catalyze a reaction that involves the oxidation of a long-chain fatty aldehyde and flavin mononucleotide, and firefly luciferase catalyzes the oxidation of the heterocyclic substrate D-luciferin in the presence of ATP. The features common to different luminescence systems are the requirement of oxygen and the ability to emit visible light of different wavelengths. As a reporter, the firefly luciferase gene (*lucFF*) (6) has advantages over the bacterial luciferase genes (*luxAB*) (4, 13). Firefly luciferase has a quantum yield of about 90% (i.e., efficiency of conversion of chemical energy to light energy) during catalysis, whereas that of bacterial luciferase is only about 5 to 10% (19, 20).

Here we report the construction of luminescent bacterial strains for the measurement of arsenite and antimonite. The sensor plasmid is based on a reporter gene coding for firefly luciferase and on the regulation unit from the *ars* operon of plasmid pI258 from multiple-metal-resistant *Staphylococcus aureus* (12). The regulation unit consists of the *ars* promoter and the repressor protein, ArsR. In the absence of arsenite, antimonite, and cadmium, the expression of the *lucFF* gene is repressed. By the addition of these metals, gene expression is induced and light emission can be quantitated.

MATERIALS AND METHODS

Materials. Tryptone, yeast extract and Casamino Acids were obtained from Difco Laboratories. D-Luciferin was obtained from Bio-Orbit Oy (Turku, Finland). $NaAsO_2$, Na_2HAsO_4, $FeSO_4$, and Li_2SO_4 were obtained from Sigma; $HgCl_2$, $CdCl_2$, $CuSO_4$, $CoCl_2$, and $MnCl_2$ were obtained from Riedel-de Haën; $ZnCl_2$, $SnCl_2$, and $NiCl_2$ were obtained from Merck; $C_4H_4KO_7Sb$ was obtained from Fluka; and $Pb(CH_3COO)_2$ was obtained from J. T. Baker. DNA-modifying enzymes were either from Promega or New England Biolabs. Vent DNA-polymerase was from New England Biolabs. All chemicals were of analytical grade.

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. The plasmid-containing strains were maintained on L agar plates supplemented with 30 μg of kanamycin per ml.

Construction of plasmid pTOO21. Plasmid pTOO21 is a p602/22-based (18) shuttle vector in which firefly luciferase (*lucFF*) expression is controlled by the *ars* promoter from *S. aureus* plasmid pI258 (Fig. 1). pTOO21 was constructed by standard recombinant-DNA techniques (25) as follows. The *arsR* gene and promoter/operator of the *ars* operon were isolated by PCR (24), and the *Bam*HI and *Xho*I restriction sites were generated with the following oligonucleotide primers: 5′-ATAT**CTCGAG***TAAAATAACATAGACAATAATCT*-3′ (at the beginning of the *arsR* gene) and 5′-TTAA**GGATCC***CCCTCATCAACAGTCACCT GATT*-3′ (at the end of the *arsR* gene). (The *Xho*I and *Bam*HI restriction sites,

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Reference or source
Strains		
E. coli MC1061	*c*I$^+$ Δ(*ara leu*)*7697* Δ*lacX74 galU galK hsr hsm*$^+$ *rpsL araD139*	2
B. subtilis BR151	*lys-3 metB10 trpC2*	39
S. aureus		
NCTC 50581	Containing plasmid pI258	21
RN4220	Efficient acceptor of *E. coli* DNA	16
Plasmids		
pTOO21	*ars* promoter and *arsR* of pI258 cloned into pCSS810 upstream of *lucFF* gene	This work
pCSS810	*E. coli-B. subtilis* shuttle vector, T5 promoter-*lac* operator upstream of *lucFF* gene, resistance to kanamycin and chloramphenicol	17

respectively, are in bold-faced type, and the bases corresponding to the *arsR* gene are italicized.) Plasmid pI258 isolated from *S. aureus* (NCTC 50581) was used as a template for the PCR. The generated fragment included at the 5′ end of the *ars* 3 bases from the beginning of the *lucFF* gene because the *Bam*HI site was originally engineered inside the luciferase gene (17). The PCR product was purified with the QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany) and digested with the restriction enzymes *Bam*HI and *Xho*I. The resulting 380-bp fragment was purified from an agarose gel by QIAquick gel extraction kit (Qiagen GmbH). The fragment was ligated with *Bam*HI- and *Xho*I-digested and phosphatase-treated pCSS810 (17) and transformed into *Escherichia coli* strain MC1061 cells by electroporation (7). Plasmid pTOO21 was isolated, and its structure was confirmed by restriction enzyme digestion and by sequencing, after which it was transformed by electroporation into *S. aureus* strain RN4220 (26) and *Bacillus subtilis* strain BR151 (35). *S. aureus* strain RN4220, harboring the parental plasmid pCSS810, was used as a control, since in pCSS810 the expression of *lucFF* is controlled by phage T5 promoter and *lac* operator and is thus independent of the concentration of metals used.

Cultivation of bacteria. Bacteria were cultivated in Luria-Bertani medium (10 g of tryptone–5 g of yeast extract–5 g of NaCl per liter [pH 7.0]) supplemented with 30 μg of kanamycin per ml in a shaker at 37°C. The overnight culture of the bacteria was harvested by centrifugation at optical densities at 600 nm of 4 to 6 for *S. aureus*, 3 for *E. coli*, and 4 to 5 for *B. subtilis. S. aureus* and *E. coli* cells were washed twice with M9 medium (25) supplemented with 0.5% Casamino Acids, and *B. subtilis* cells were washed with Spizizen's minimal medium (9) supplemented with 0.5% Casamino Acids. Bacteria were suspended and diluted with the same media before the measurements. About 10^6 *S. aureus* cells, 3×10^6 *E. coli* cells, and 10^6 *B. subtilis* cells were used per measurement.

Luminescence measurements. The following salt solutions: $NaAsO_2$, $C_4H_4KO_7Sb$, $CdCl_2$, Na_2HAsO_4, $ZnCl_2$, $SnCl_2$, $HgCl_2$, $FeSO_4$, $Pb(CH_3COO)_2$, Li_2SO_4, $CuSO_4$, $MnCl_2$, $NiCl_2$, and $CoCl_2$ were made in distilled water. Different dilutions of these solutions were pipetted to the wells of a 96-well white microtitration plate (Labsystems, Helsinki, Finland) in a volume of 50 μl. The same volume of one of the above-mentioned bacterial dilutions was added, and the plate was incubated without shaking at 30°C. *S. aureus* strain RN4220(pTOO21) was incubated for 120 min, *E. coli* MC1061(pTOO21) was incubated for 90 min, and *B. subtilis* BR151(pTOO21) was incubated for 240 min. Then the plate was moved to a Luminoskan luminometer (Labsystems), 100 μl of luciferase substrate (1 mM D-luciferin in 0.1 M Na citrate buffer, pH 5.0) was added through the dispenser, and in vivo bioluminescence peak values were measured immediately. All measurements were done in triplicate.

Induction coefficiencies were calculated to define inducing efficiencies with different metals as follows: induction coefficient $I = I_{I/I_B}$, where I_I is the maximal light emitted by the induced sample and I_B is the light emitted by the uninduced sample, which is the background light.

Freeze-drying of strain RN4220(pTOO21). An overnight culture was diluted 1:50 into 50 ml of Luria-Bertani medium supplemented with 30 μg of kanamycin per ml and grown in a shaker at 37°C to an optical density at 600 nm of 4. Cells were harvested by centrifugation and suspended in the same volume of M9 medium supplemented with 10% lactose. Cells were freeze-dried in 200-μl aliquots by standard procedures (11, 31). Freeze-dried cells were tested by induction measurements with arsenite. Cells were reconstituted in 200 μl of distilled water, and luminescence measurements were done in the same way as with fresh cells.

RESULTS

Construction of the plasmid pTOO21. The original *lac* operator/T5 promoter element controlling the luciferase gene expression of pCSS810 (17) was replaced by the arsenite-responsive regulation unit (Fig. 1). The cloned unit contained the *arsR* gene, which encodes the regulatory protein (12, 23), and the *ars* promoter, from which *arsR* and the structural *ars* genes in pI258 are transcribed (12). In the absence of arsenite, the expression level of luciferase was low in *E. coli* and *S. aureus*. In addition, the expression of the chloramphenicol acetyltransferase (*cat*) gene, which is located downstream from the luciferase gene in the plasmid construct, was probably repressed, because *S. aureus* RN4220(pTOO21) cells were not able to grow in the presence of 30 μg of chloramphenicol per ml in contrast to *S. aureus* RN4220(pCSS810) (data not shown).

Luminescence measurements. Luminescence measurements were optimized to detect arsenite with a simple measurement protocol. Induction conditions, such as incubation time, incubation temperature, amount of sensor bacteria per measurement, growth phase of bacteria, and incubation medium, were varied. Also, different host bacteria, *E. coli* MC1061 and *B. subtilis* BR151, were tested, and optimization of induction conditions was repeated with each strain. Conditions which affected induction with *S. aureus* RN4220(pTOO21) were the growth phase of the sensor bacteria and the time used to incubate bacteria with metals (Fig. 2). Although the amount of light emitted was still increasing after 2 h of incubation, the induction coefficient did not increase considerably after 70 min, and sensitivity did not improve (data not shown). Late logarithmic and stationary growth phase of the bacteria improved the shape of the curve by extending the concentration range to where arsenite can be detected (data not shown) with *S. aureus*. Other alterations had no noticeable effect on the measurement. Results with the two other host strains showed that induction time had an effect on induction profiles. Furthermore, the optimal induction time was different for each strain (data not shown), so the optimal induction time of the particular strain was used in measurements.

Induction of *S. aureus* RN4220(pTOO21) with different compounds. The following oxyanions caused induction of luminescence: arsenite (AsO_2^-), antimonite (SbO_2^-) (Fig. 3A and B), and arsenate (AsO_4^{3-}). Interestingly, the presence of cadmium (Cd^{2+}) (Fig. 3C) cations also caused a considerable increase in luminescence, and zinc (Zn^{2+}) resulted in a weak induction (data not shown). The lowest arsenite concentration that caused a noticeable induction [(background + ($2 \times$ standard deviation)] was 100 nM (Fig. 3A). Luminescence increased with increasing amount of AsO_2^- to a concentration of 3.3 μM; at concentrations from 3.3 to 100 μM, luminescence was at a constant level, after which luminescence began to fall

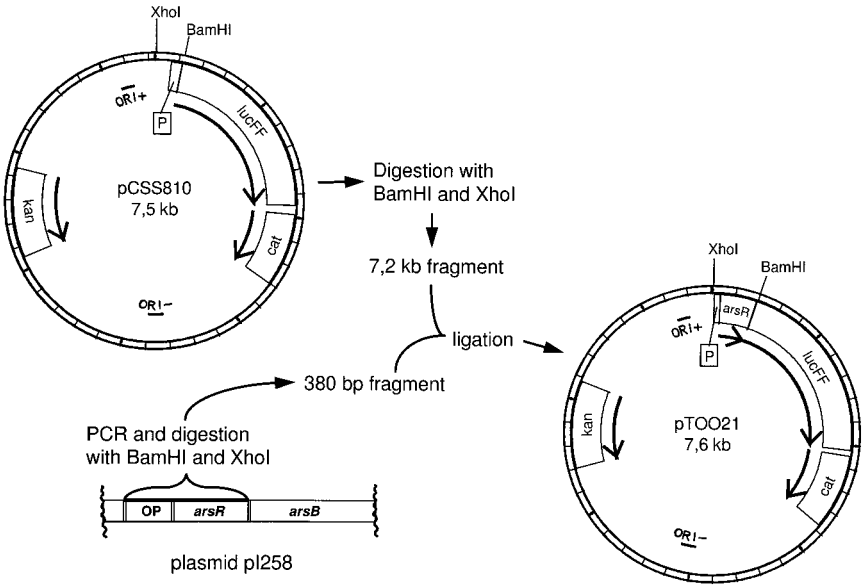

FIG. 1. Construction of the plasmid pTOO21. Plasmid pCSS810 was digested with *Bam*HI and *Xho*I, and the resulting 7.2-kb fragment was isolated and treated with calf intestine phosphatase. A 380-bp fragment was amplified from plasmid pI258 by PCR with the primers described in Materials and Methods. The 380-bp fragment was purified, cut with *Bam*HI and *Xho*I, purified, and ligated with the 7.2-kb *Bam*HI-*Xho*I fragment from pCSS810. Abbreviations: *kan*, gene encoding kanamycin resistance; *cat*, gene encoding chloramphenicol resistance; *arsR*, gene encoding regulative protein of the *ars* promoter; P, promoter, *lucFF*, gene encoding firefly luciferase; ORI+ and ORI−, origins of replication in gram-positive and gram-negative bacteria, respectively.

and reached background values at 10 mM, resulting in a bell-shaped curve. Concentrations needed for luminescence with SbO_2^- ions were slightly lower than with AsO_2^- ions (Fig. 3B). Increasing SbO_2^- concentration increased luminescence from 33 nM to 3.3 μM, after which luminescence began to fall. Luminescence reached background values at 3.3 mM. Cd^{2+} induced a somewhat different luminescence response (Fig. 3C), resulting in quite a sharp peak at concentrations from 330 nM to 33 μM. Despite the differences in luminescence curves of these metals, all reached their maximum luminescence at a concentration of 3.3 μM. The maximum induction coefficients for arsenite, antimonite, and cadmium were 50, 54, and 23, respectively. Na_2HAsO_4 and $ZnCl_2$ induced minor luminescence at concentrations greater than 100 μM, with maximum induction coefficients of about 2. $SnCl_2$, $HgCl_2$, $FeSO_4$, $Pb(CH_3COO)_2$, Li_2SO_4, $CuSO_4$, $MnCl_2$, $NiCl_2$, and $CoCl_2$ did not cause significant luminescence, even at millimolar concentrations (data not shown).

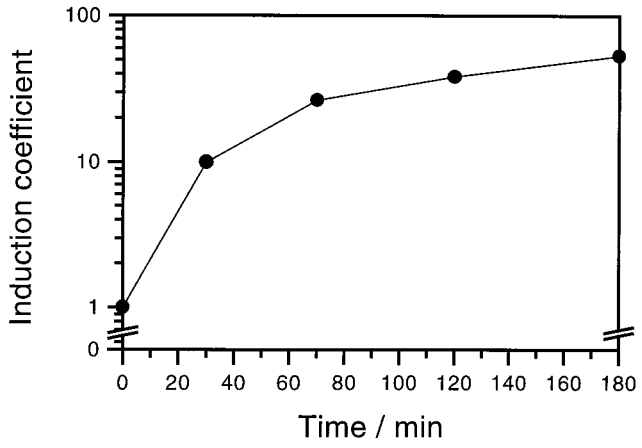

FIG. 2. Effect of incubation time on induction. The induction coefficients obtained for arsenite- and antimonite-sensing bacterial strain *S. aureus* RN4220(pTOO21) when incubated with 10 μM $NaAsO_2$ at 30°C at different time points are shown. Luminescence measurements were done as described in Materials and Methods. The induction coefficient was calculated as the luminescence ratio between induced and uninduced cells as described in Materials and Methods.

S. aureus RN4220(pTOO21) cells were freeze-dried and found to be well preserved and viable. Luminescence of freeze-dried cells was induced with AsO_2^- (Fig. 4). The sensitivity remained the same as with freshly cultivated cells, but the induction coefficient decreased to about 10.

Induction of *B. subtilis* BR151(pTOO21) with different compounds. Metals which induced this strain were arsenite (Fig. 5), arsenate, antimonite, and cadmium. The lowest arsenite concentration which caused the induction of luminescence was 3.3 μM; light emission increased with higher AsO_2^- concentrations to a concentration of 100 μM, after which light emission dropped and reached background values at 3.3 mM. AsO_4^{3-} induced light production at concentrations from 330 μM to 10 mM. SbO_2^- induced light production at 330 nM to 330 μM. Cd^{2+} had only a minor effect on light induction at 330 nM concentration (data not shown). The maximum induction coefficients were 24, 5, 20, and 2, respectively.

Induction of *E. coli* MC1061(pTOO21) with different compounds. Luminescence was induced with AsO_2^-, AsO_4^{3-}, SbO_2^-, and Cd^{2+}. Concentrations needed for induction were 3.3 to 330 μM for AsO_2^- (Fig. 5), 33 μM to 3.3 mM for AsO_4^{3-}, 3.3 to 33 μM for SbO_2^-, and 33 μM to 3.3 mM for Cd^{2+}. The maximum induction coefficients were about 6, 4, 2, and 2, respectively (data not shown).

The effect of various metals on the constitutive light-producing strain *S. aureus* RN4220(pCSS810). The control strain with constant light-producing capability was used for measuring nonspecific toxic effects. Luminescence was stable at low AsO_2^- concentrations (Fig. 3A). At AsO_2^- concentrations of

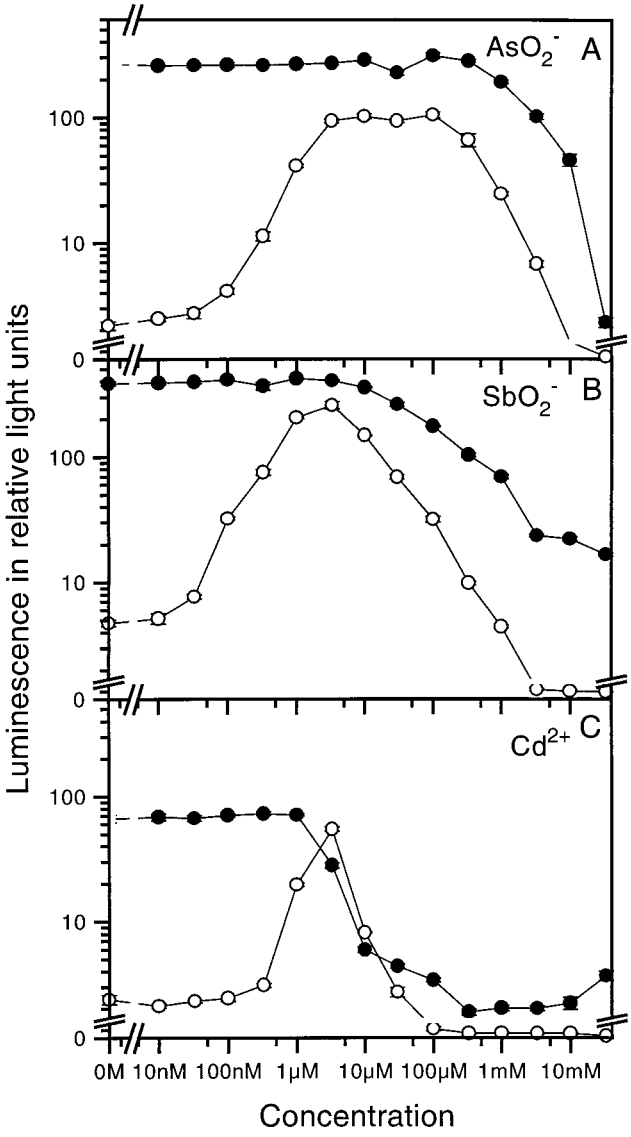

FIG. 3. Light emission by the bacterial strains. Measurements for luminescence produced by arsenite- and antimonite-sensing strain *S. aureus* RN4220 (pTOO21) (○) and by stable light-producing control strain RN4220(pCSS810) (●) incubated with (A) $NaAsO_2$, (B) $C_4H_4KO_7Sb$, or (C) $CdCl_2$ at 30°C for 2 h are shown. Data represent means ± standard deviations from three determinations. Error bars are shown only when they exceed the size of the circles. Luminescence measurements were done as described in Materials and Methods.

1 to 33 mM, luminescence decreased. The fall in luminescence caused by SbO_2^- began at a concentration of 10 μM (Fig. 3B). Cd^{2+} caused a gradual decrease in luminescence in the concentration range from 1 to 330 μM (Fig. 3C).

DISCUSSION

The recombinant bacterial strain *S. aureus* RN4220 (pTOO21) described here responds to arsenite, antimony, and somewhat surprisingly, cadmium. The luminescence curve for the induction with Cd^{2+} is different from those of SbO_2^- and AsO_2^- (Fig. 3). The concentration range in which induction occurs is narrow, probably mostly due to the fact that Cd^{2+} is more toxic than Sb and As oxyanions. The toxic effects were confirmed with a constant-light-emitting *S. aureus* RN4220

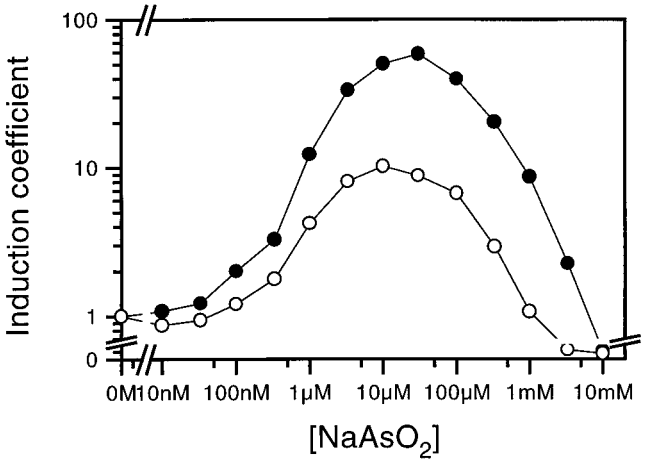

FIG. 4. Induction of light emission of freeze-dried cells. Induction coefficients of freeze-dried RN4220(pTOO21) cells (○) and of fresh RN4220 (pTOO21) cells (●) incubated with $NaAsO_2$ at 30°C for 2 hours are shown. Error bars are shown only when they exceed the size of the circles. Luminescence measurements were done as described in Materials and Methods. The induction coefficient was calculated as the luminescence ratio between induced and uninduced cells as described in Materials and Methods.

harboring the parental plasmid pCSS810 as a control strain. It is analogous to conventional toxicity test strains: light production is stable until the toxic effect of the metals causes a reduction in light production (1, 22). There is a direct correlation between viable microbial cell count and luciferase activity in vivo (36). The use of a constant-light-producing strain, in addition to a specific inducible strain, ensures the correct estimation of sample toxicity.

The sensitivity of metal detection obtained here exceeded the sensitivities of two earlier reported sensor strains (5, 12) which were used to study the regulation of the arsenic resistance operon and was of the same order of sensitivity level as that of a similar sensor bacteria strain (27). Corbisier et al. (5)

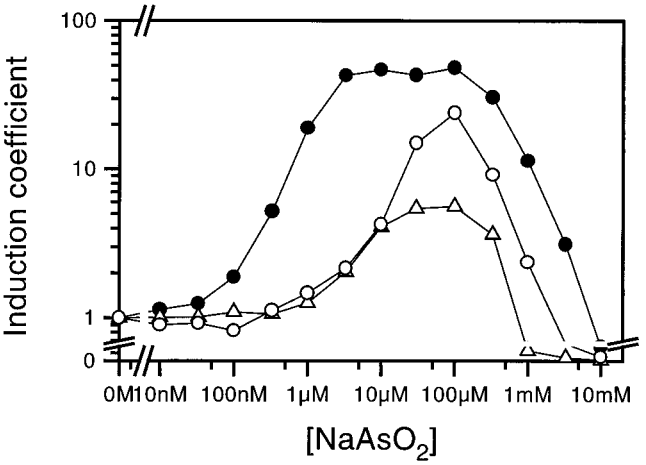

FIG. 5. Comparison of induction of luminescence by different bacterial strains. Induction coefficiencies obtained with strains *S. aureus* RN4220 (pTOO21) (●), *E. coli* MC1061(pTOO21) (△), and *B. subtilis* BR151(pTOO21) (○) are shown incubated with $NaAsO_2$ at 30°C for 2, 1.5, and 4 h, respectively. Error bars are shown only when they exceed the size of the symbols. Luminescence measurements were done as described in Materials and Methods. The induction coefficient was calculated as the luminescence ratio between induced and uninduced cells, as described in Materials and Methods.

used an *arsB-luxAB* fusion, and Ji and Silver (12) used an *arsB-blaZ* fusion, with the regulatory region originating from plasmid pI258. *S. aureus* RN4220 was used as the host strain in both cases. Scott et al. (27) used an *arsR-lacZ* fusion with the regulatory region originating from plasmid R773 and *E. coli* JM109 as a host strain. The detection limits in the studies of Corbisier et al. and Ji et al. were about 1 μM or higher for arsenite and 330 nM for antimonite (over 10 times the detection limits obtained here). The detection limits in Scott's study were 100 nM for both arsenite and antimonite, but a 17-h induction period was needed to achieve this result, compared to 2-h incubation periods in this study. There are few possible explanations why the bacterial strain shown in this study is more sensitive than the above-mentioned strains. One explanation is the difference in the sensitivity of detection between firefly luciferase (used here) and bacterial luciferase (used by Corbisier et al. [5]). The reporter gene *lacZ*, used by Scott et al., lacks sensitivity, partly because this gene is naturally present in bacterial cells (27), and therefore, the background activity is high. On the other hand, because Scott's sensor bacteria were as sensitive as our strain, it is possible that the regulatory protein ArsR of the *ars* operon of plasmid R773 is activated by a smaller ion concentration than that of plasmid pI258. The background (uninduced) enzyme activity level varies for the different strains and regulators, and the intracellular metabolism could also differ. For example, the mercury-sensing bacteria recently reported by us (37) had a higher sensitivity than an analogously constructed bacterial strain (28) which utilized *lux* genes. However, in the case of mercury, the difference in sensitivity was almost a millionfold compared to a difference of less than a hundredfold obtained here with arsenite sensors. On the other hand, the mercury sensor strain is significantly more sensitive than the arsenite sensor bacteria described here, and huge differences in the sensitivities of sensors for different metals make comparison unreasonable. For example, mercury is significantly more toxic than arsenite, and this has a great impact on the detection limits. This result also makes sense from an evolutionary point of view.

Patterns of induction differ slightly from those observed in earlier reports. Corbisier et al. (5) found that only arsenite induced luminescence, whereas Ji and Silver (12) reported strong induction with arsenate among other ions (AsO_2^-, SbO_2^-, and Bi^{3+}). Our results fall in between these two reports, with the finding of only slight induction by arsenate. However, our most noticeable finding was the induction of luminescence by cadmium, which has not been reported previously. The range of inducers is affected by differences in intrinsic cation and oxyanion concentration and transport systems between bacterial hosts. The sensor strain of Scott et al. (27) was based on another arsenic operon, from R773 of *E. coli*, but the induction pattern was similar to that of our strain except that it excluded cadmium, which they did not test. The induction with cationic Cd^{2+} seems to be quite surprising at first; however, it has been found previously that Bi^{3+} is also an inducer of the *ars* operon (12). Studies of Shi et al. (29, 30) show that the ArsR protein of R773 is activated by As^{3+} or Sb^{3+} rather than by the oxyanion AsO_2^- or SbO_2^-. Furthermore, the ArsR proteins of R773 and pI258 belong to the same family of metalloregulatory proteins, the ArsR family, with the cadmium regulatory protein, CadC, of pI258 (32). All proteins of the ArsR family have the same highly conserved region, which is proposed to be the metal binding region (30).

We also wanted to determine if plasmid pTOO21 could be used in host strains other than *S. aureus*. Plasmid pTOO21 was transformed into *B. subtilis* BR151 and *E. coli* MC1061. Neither strain was as good as *S. aureus* for sensitivity and for inducing efficiencies (Fig. 5). It could be that ArsR protein does not repress gene expression as well in *B. subtilis* and *E. coli* and that an increased basal level of luciferase expression causes a decrease in the induction coefficient and in sensitivity. In an uninduced state, both *B. subtilis* and *E. coli* cells emit considerably more light per cell than *S. aureus* does (data not shown).

Our data also indicate that the use of reagent-like freeze-dried bacteria can replace the routine cultivation of bacteria. Although the inducing efficiency of the cells is somewhat lowered, the sensitivity of the cells is similar to that of fresh cells (Fig. 4). Further optimization of freeze-drying conditions might also improve inducing efficiency.

Arsenic toxicity depends on its oxidation state, as summarized by Cervantes et al. (3). Arsenite is about 100-fold more toxic than arsenate. Arsenic occurs in soil as arsenite and arsenate and their methylated derivatives as a result of microbial activity. Arsenite is also the main arsenic compound in seawater. In contrast to routine analysis of total arsenic, the sensor bacteria described here primarily measure bioavailable arsenite, the most toxic form of arsenic. However, quantification of bioavailable arsenite does not replace traditional chemical analysis; rather, these sensor bacteria can complement analytical chemical methods in the detection and screening of biologically available arsenite in environmental samples. A comparison of the bioavailability of a metal with its total metal content allows the examination of an inert metal. This can be helpful in the planning and controlling of the bioremediation process.

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