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Measurement of Firefly Luciferase Reporter Gene Activity from Cells and Lysates Using *Escherichia coli* Arsenite and Mercury Sensors

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The structural gene encoding firefly luciferase from *Photinus pyralis* is a widely used reporter both in traditional monitoring of gene expression and in bacterial sensors. Its activity can be detected from living cells (*in vivo*) without disruption or from cell-free lysate (*in vitro*). We compared the two measurement methods by using an overall toxicity detecting strain *Escherichia coli* MC1061(pCSS810), a mercury-sensing strain *E. coli* MC1061(pTOO11), and two new arsenic sensor strains MC1061(pTOO31) and AW3110(pTOO31) which were constructed for this study. Plasmid pTOO31 was constructed by inserting the *ars* promoter and the *arsR* gene from plasmid R773 to control firefly luciferase gene expression. Both *in vivo* and *in vitro* methods correlated well with the strains tested [correlation coefficients $R = 0.99484$ and 0.99834] and gave highly comparable results with standard solutions of arsenite or mercury ions and from six environmental water samples spiked with the ions. Use of the *in vivo* method resulted in lower variation between replicates of the same sample (CVs ranging from 3.9 to 7.2%) and also between different samples (from 8.6 to 25.9%) compared to the *in vitro* method (CVs ranging from 8.6 to 17.8% for replicates and from 13.1 to 36.3% for different samples).

Key Words: **bioluminescence; heavy metal; arsenic ions; *luc;* sensor bacteria.**

Luminescence and fluorescence are emerging techniques to measure gene expression in both a traditional sense (i.e., in the determination of the activity of various promoters under different physiological conditions) and bacterial sensor use. The most often used reporter gene with luminescence as a detected signal is *luc* encoding firefly, *Photinus pyralis*, luciferase. This luciferase gene can be expressed in a wide variety of host cells from prokaryotes (1) to plants (2) and mammalian cells [reviewed in (3) and (4)]. Close homologues of firefly luciferase, luciferases from a click beetle *Pyrophorus plagiophthalamus*, are capable of producing light of different colors ranging from green (wavelength maximum at 547 nm) to orange (593 nm) (5, 6) and the corresponding genes have been cloned (7). The detection of both *P. pyralis* and *P. plagiophthalamus* luciferases is possible without disruption of the cells because the substrate of those enzymes, D-luciferin, passes through cell membranes at acidic pH (8). Another luminescence reporter gene system, *luxAB* encoding bacterial luciferase, is generally restricted to bacteria because the native enzyme consists of two different polypeptides [for a review see (9)]. The expression of the whole bacterial luciferase operon produces a self-luminescent cell without any additions allowing thereby the real-time monitoring of gene expression, whereas the expression of only the *luxA* and *luxB* genes that encode luciferase polypeptides requires the externally added substrate (a long-chain aldehyde) for luminescence. Yet another reporter, green fluorescent protein (GFP),[2] has gained widespread interest especially in studies concerned with localization or timing of expression in eukaryotic cells [reviewed in (10)]. GFP can be detected in real time without addition of any substrates due to its intrinsic fluorescent properties. The problem with GFP is the background fluorescence of cells or any material present (such as plastics used) while excited.

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[2] Abbreviations used: GFP, green fluorescent protein; *R*, correlation coefficient; CV, coefficient of variation.

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Several whole-cell sensors carrying luciferase genes, either bacterial (11, 12) or firefly (13, 14), under the control of heavy-metal-responsive elements have been described. These elements originate from microbes living in contaminated soil or water. These microbes have developed precise controlling mechanisms during evolution for the detoxification and/or efflux of toxic metals from the cell [for an excellent recent review, see (15)]. These microbial sensors commonly detect very low levels of metals or metalloids, down to attomole levels, and the metal detected must be bioavailable which is a unique criterion that is very difficult or even impossible to measure with the means of conventional analytical chemistry.

In this study, we compare the two methods of measuring firefly luciferase activity in bacteria: direct real-time measurement of luminescence from living cells and measurement of luciferase activity from a cell-free lysate. In principle, the use of a cell lysate (*in vitro* method) could have advantage over use of living cells (*in vivo* method) because the reaction conditions are optimized in commercial luciferase assay reagents, which are available from several manufacturers. Furthermore, the measured luminescence could correlate better with the amount of luciferase enzyme present inside the cells using the *in vitro* method, because the cell membrane forms a barrier for the luciferase substrate, D-luciferin, and the luminescence could therefore be dependent more on the diffusion of luciferin than on the amount of luciferase. On the other hand, the contents of the cell provide all the necessary additional substrates and cofactors needed in a well-buffered and rather constant environment, which would mean that the conditions for the luciferase reaction remain relatively unchanged. The *in vivo* method does not require the purchase of expensive commercial reagents either and it also requires fewer pipetting steps in practice.

As test bacteria we used one strain with constitutive firefly luciferase production and three metal-sensing bacteria equipped with inducible promoters, one responding to mercury (13) and the other two to arsenic compounds (constructed in this study). To compare the *in vivo* and *in vitro* luciferase activity measurements we used a recently described chemical disruption method developed especially for *Escherichia coli* cells (16). To our knowledge this is the first time this kind of direct comparison has been made despite the importance of the choice of method in both sensor bacteria and gene expression studies.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Bacterial strains and plasmid used in this study are listed in Table 1. The sensor plasmid pTOO31 was constructed by similar protocols described earlier for other metal sensors (13, 14, 17). Standard recombinant DNA methods described previously (18) were used. In short, *ars* regulatory region and adjacent regulator structural gene (*arsR*) were amplified by polymerase chain reaction (19) from *E. coli* plasmid R773 (20) (GenBank Accession No. X16045) using primers 5′-ATAT**CTCGAG**CAAGTTATCTCACCTACCTTAA-3′ and 5′-TTAA**GGATCC**CCTCATCTTAAATACCTCGCATTT-3′ (restriction sites for *Xho*I and *Bam*HI shown in bold). PCR was carried out using Vent DNA polymerase from New England Biolabs (Beverly, MA). The PCR fragment and plasmid pCSS810 (1) were digested with *Xho*I and *Bam*HI and purified using agarose gel electrophoresis and a Qiagen gel extraction kit (Hilden, Germany). They were ligated together using T4 DNA ligase and the ligation mixture was electroporated (21) into *E. coli* MC1061 cells. The correct transformants were selected by their ability to produce luminescence that was induced by the addition of 1 μM arsenite. The resulting plasmid, pTOO31, was purified using a Qiagen plasmid purification kit (Hilden, Germany) and its structure confirmed by multiple restriction enzyme digestion. Plasmid pTOO31 was also transformed into strain *E. coli* AW3110 which has a deletion in its chromosomal arsenite resistance operon (22) for testing if the sensitivity of the sensor would be increased because of the accumulation of arsenite ions inside cells.

TABLE 1

Bacterial Strains and Plasmids

Strain or plasmid	Description	Source
	Strains (all *Escherichia coli*)	
AW3110	K12 F^- IN(*rrnD-rrnE*) Δ*ars::cam*	(22)
MC1061	*ara*D139, Δ(*ara-leu*)7697, Δ*lacX74*, *galU*, *galK*, *hsdR2* (r_K-m_K), *mcrB1.*, *rps*L(str^R), cI^+	(25)
	Plasmids	
pCSS810	*E. coli–Bacillus subtilis* shuttle vector, T5 promoter–*lac* operator upstream of the *luc*FF gene, kanamycin and cloramphenicol resistances	(1)
pTOO11	*mer* promoter and *merR* of transposon Tn21 inserted into pCSS810 upstream of the *luc*FF gene	(13)
pTOO31	*ars* promoter and *arsR* of R773 inserted into pCSS810 upstream of the *luc*FF gene	This study

Cultivation of Cells

E. coli cells were cultivated overnight in 5 ml of LB medium (10 g Bacto tryptone, 5 g yeast extract, 5 g NaCl, pH 7.0) (18) containing 30 μg/ml kanamycin at

37°C. Main cultures were done by inoculating 0.5 ml of the overnight culture into 50 ml of the same medium and grown at 37°C to an optical density of 0.8–1.2 at 600 nm. Cells were washed twice and suspended in the original volume of M9 medium (18) containing 0.5% casein hydrolysate. Cells were diluted in the same medium one to a hundred, giving a final concentration of about 1×10^6 cells per measurement.

Freeze-Drying of Cells

Cells were cultivated for freeze-drying in the same way as for direct measurements. Harvested cells were washed with and suspended in M9 medium containing 10% lactose. Freeze-drying was performed according to standard procedures (23, 24) in 200 μl or 1-ml aliquots using a Lyoflex 10 freeze-dryer (Edward's, Crawley, UK). Cells were reconstituted by adding 200 μl or 1 ml of distilled water, respectively, and luminescence measurements were done as with fresh cells.

Lysis of Cells

Lysis of *E. coli* cells was done using a 2× lysis buffer which had the following composition: 200 mM Tris–HCl, pH 7.8; 64 mM NaH_2PO_4; 16 mM dithiothreitol; 16 mM CDTA (*trans*-1,2-diaminocyclohexane *N,N,N′,N′*-tetraacetic acid); 8% Triton X-100; 20% glycerol; 50 μg/ml polymyxin β sulfate (16). This buffer was mixed with an equal amount of cells in M9 medium. The lysis was practically instantaneous and there was no need for centrifugation of cell debris for this application.

Activity Measurement in Vitro

In vitro measurement of luciferase was performed using a commercial kit capable of measuring low levels of insect luciferase activity [GenGlow kit supplied by BioOrbit Oy (Turku, Finland)]. White 96-well plates were used and 20 μl of the lysed cells (corresponding to approximately 2×10^5 cells) was added per well. The plate was inserted into a Luminoskan luminometer (Labsystems Oy, Helsinki, Finland), the temperature was stabilized to 30°C, and 100 μl of the GenGlow reagent was added using the dispenser of the device. The peak luminescence was measured for 5 s immediately after dispension.

The stability of luminescence *in vitro* after substrate addition was measured with strain MC1061(pTOO31): After the initial *in vitro* measurement with substrate addition, luminescence measurement was repeated from the same sample without further addition of substrate at various time points up to 55 min.

Activity Measurement in Vivo

In vivo luminescence was measured from an induction reaction mixture (containing 50 μl metal or oxyanion solution and 50 μl cells) by adding 100 μl of 1 mM D-luciferin (BioOrbit Oy) in 100 mM Na–citrate buffer, pH 5.0, with the dispenser of the luminometer. Luminescence was measured using a Luminoskan plate luminometer (Labsystems Oy) at 30°C. The reactions were monitored as described above in activity measurements *in vitro*.

Comparison of in Vitro and in Vivo Measurements

Diluted MC1061(pCSS810), MC1061(pTOO11), and MC1061(pTOO31) cells were divided into two batches. One was measured for *in vivo* luminescence by the addition of D-luciferin in citrate buffer and the other was lysed with lysis reagent. After lysis, the lysate was measured for *in vitro* activity using GenGlow reagent.

Stability Measurements of Luciferase in Cell Lysate

The stability of luciferase in cell lysate was measured using two *E. coli* strains, MC1061(pCSS810) and MC1061(pTOO31). MC1061(pTOO31) cells were induced with two concentrations of sodium arsenite (0.1 and 10 μM) for 90 min and cells were lysed thereafter, whereas MC1061(pCSS810) cells were lysed immediately after cultivation. A 20-μl sample of the lysate was immediately assayed for luciferase produced as described previously. Lysed cells were incubated at room temperature and *in vitro* measurements were repeated four times during an incubation period of 60 min.

Induction with Different Compounds

Metal and oxyanion dilutions or spiked water samples were pipetted onto white 96-well Cliniplates (Labsystems Oy) in a volume of 50 μl. The same volume of diluted cells was added and plates were incubated for 90 min with strains MC1061(pTOO31) and AW3110 (pTOO31) or 60 min with strain MC1061(pTOO11) at 30°C without shaking. Both stock solutions and dilutions (from noninducing low nanomolar to highly toxic millimolar concentrations) were done in commercial toxic substance-free water (Pharmacia, Uppsala, Sweden). Metals and oxyanions used were $NaAsO_2$, Na_2HAsO_4, $FeSO_4$, Li_2SO_4, $HgCl_2$, $CdCl_2$, $CuSO_4$, $CoCl_2$, $MnCl_2$, $ZnCl_2$, $SnCl_2$, $NiCl_2$, $C_4H_4KO_7Sb$, and $Pb(CH_3COO)_2$. These compounds were purchased from Sigma, Riedel-de Haën, Merck, Fluka, and J. T. Baker. All metals and metalloids were of analytical grade, except $C_4H_4KO_7Sb$, which was pure grade (≥99%).

Calculations of Induction Coefficients

Induction coefficients were calculated with the formula $I = L_i/L_b$, where I is the induction coefficient, L_i is the luminescence value from the sample, and L_b is

TABLE 2

Induction of Strain MC1061(pTOO31) with Different Compounds

Inducing compound	Range of detection	Maximum induction	Induction coefficient
[$NaAsO_2$]	33 nM–1 mM	10 μM	45
[Na_2AsO_4]	33 μM–33 mM	0.3–1 mM	35
[$C_4H_4KO_7Sb$]	100 nM–100 μM	10 μM	30
[$CdCl_2$]	10 μM–10 mM	100 μM–10 mM	10

Note. Solutions of compounds were made into commercial water and measured as described under Materials and Methods. The induction coefficient, defined under Materials and Methods, is of maximum induction.

the luminescence value from blank noninducing solution using L_i and L_b from the same time point.

Water Samples

Six water samples from different sources were used in order to test the reproducibility of the *in vivo* and *in vitro* methods under different reaction conditions. Water samples were spiked with different arsenite or mercury concentrations and measured for inducibility with strains MC1061(pTOO31), AW3110(pTOO31), and MC1061(pTOO11) and for toxicity with strain MC1061(pCSS810) as described above.

Samples were (i) well water from the countryside having some salt as contaminant, (ii) river water from which the local communal water is purified, (iii) brackish seawater, (iv) tap water from our department, (v) tap water from a small village having their communal water from a natural fountain, and (vi) well water from an area which is expected to have a leak from brackish seawater.

RESULTS

Characterization of Arsenic Sensor Strains

The *ars* regulatory region originating from *E. coli* plasmid R773 was inserted in front of the firefly luciferase gene (*luc*) and the resulting plasmid, pTOO31, was transformed into *E. coli* strains MC1061 (25) and AW3110 (22). Induction conditions for luciferase production were optimized using strain MC1061(pTOO31) and different arsenite dilutions were made in purified water. The best results were obtained with cells grown to logarithmic phase. The variation of cell amount between 1×10^5 and 8×10^6 cells/assay and the pH value between 5.5 and 8.0 had no effect on sensitivity or induction coefficients. The time needed for maximal induction was 90 min (data not shown). The specificity of sensor cells *E. coli* MC1061(pTOO31) to metals and oxyanions is shown in Table 2 (see also below, Fig. 2). The results obtained by *E. coli* AW3110(pTOO31) were essentially similar to the results of MC1061(pTOO31).

Freeze-Drying of Cells

The sensor strain used in this study maintained its usability after freeze-drying. However, the induction coefficients and sensitivities were lowered. As an example, the induction coefficient of strain MC1061 (pTOO31) was reduced from 45 to 12 and the smallest arsenite concentration that caused induction of luminescence was 1 μM, in contrast to fresh cells which were induced already with 33 nM arsenite. The lower induction coefficient and sensitivity could be compensated for by reconstituting freeze-dried cells and incubating them overnight at room temperature in 5 ml of M9 medium, containing 0.5% casein hydrolysate and kanamycin (30 μg/ml) (data not shown). After the initial optimizations with freshly cultivated cells, evaluations were done with freeze-dried sensor cells. The following results are obtained with lyophilized bacteria.

Stability of Luciferase in Cell Lysate

The stability of the firefly luciferase was measured from *E. coli* MC1061(pCSS810) lysate. Firefly luciferase was 100% stable in the lysate during a 60-min incubation. The stability of firefly luciferase was similar with arsenic ion-specific strain MC1061(pTOO31) (data not shown).

Comparison of in Vivo and in Vitro Measurements

Luminescence results from *in vitro* and *in vivo* assays correlated well with each other with both MC1061(pCSS810) and MC1061(pTOO31) (Fig. 1). The correlation experiments resulted in *R* factors of 0.99484 and 0.99834 for strains MC1061(pCSS810) and MC1061(pTOO31), respectively. The obvious higher dynamic measurement range of the constitutive light-producing strain *E. coli* MC1061(pCSS810) is solely due to a much higher absolute light emission capacity over inducible strain MC1061(pTOO31). The absolute luminescence values obtained with MC1061 (pTOO31) from the *in vivo* and *in vitro* measurements had some differences, *in vivo* being about 3 times greater. The reason for this is partly because of the different assay protocols; the *in vitro* assay measures 10 times fewer cells. Despite this difference, it had no effect on sensitivity or induction coefficients of metal sensor assays, which depend more on induction conditions than measurement type.

The stability of *in vitro* luminescence after substrate addition was tested with strain MC1061(pTOO31). Both low and high concentrations of arsenite ions tested with strain MC1061(pTOO31) resulted in the same decay rate of *in vitro* luminescence, about 1.5%/min (data not

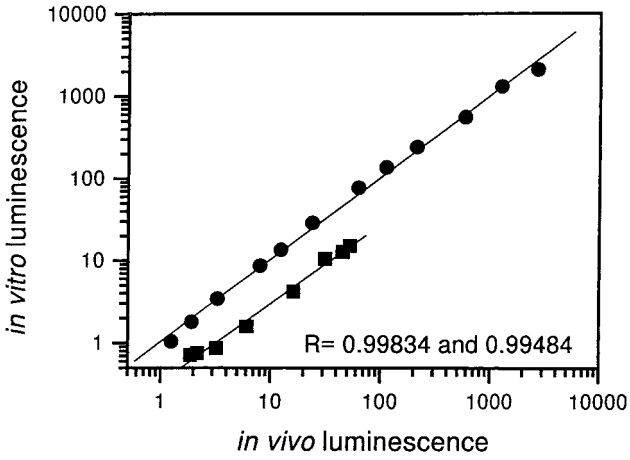

FIG. 1. Correlation between *in vivo* and *in vitro* measurements. Correlation measurements were done using two different *E. coli* strains, the arsenite sensor strain MC1061(pTOO31) (circles, R = 0.99484) and an overall toxicity detector strain MC1061(pCSS810) (squares, R = 0.99834). A dilution series of both cell types was done in LB medium supplemented with kanamycin and measurements were done *in vivo* and *in vitro*. Values from dilution series of strain *E. coli* MC1061(pTOO31) represent those of a maximally induced series with 100 μM $NaAsO_2$. Assay conditions are described under Materials and Methods.

shown), which is a typical value for the GenGlow reagent, according to the manufacturer. *In vivo* luminescence was stable between 20 and 90 min (data not shown).

Sample Measurement

All water samples were tested for their overall toxicity using strain *E. coli* MC1061(pCSS810) prior to comparison of *in vivo* and *in vitro* methods. The different samples had only minor effects on luminescence compared to the reference water; activities were from 99 to 111% (data not shown). Variations between replicates in the *in vitro* method were clearly higher with all strains (Table 3). Furthermore, variations between different samples were higher with the *in vitro* measurements than with the *in vivo* measurements (Fig. 2, Table 4 and Table 5).

TABLE 3

Variations between Replicas Using the *in Vivo* and *in Vitro* Methods

Strain	Inducer and concentrations used	*In vivo* mean CV (%)	*In vitro* mean CV (%)
MC1061(pTOO31)	Arsenite (10 nM–1 mM)	3.90	13.73
AW3110(pTOO31)	Arsenite (100 nM–100 μM)	2.29	15.51
MC1061(pTOO11)	Mercury (100 pM–100 nM)	7.24	17.79
MC1061(pCSS810)	No induction	3.94	8.56
Mean of mean CVs		4.34	13.90

Note. Mean of standard deviations (expressed as percentage of the absolute values of luminescence). Luminescence was measured as presented under Materials and Methods using three replicas and values were calculated from mean values of used metal and metalloid concentrations. Strain MC1061(pCSS810) was used with both arsenite and mercury, but this strain expresses constitutive luminescence characteristics and metals cause only a toxic effect at high concentrations.

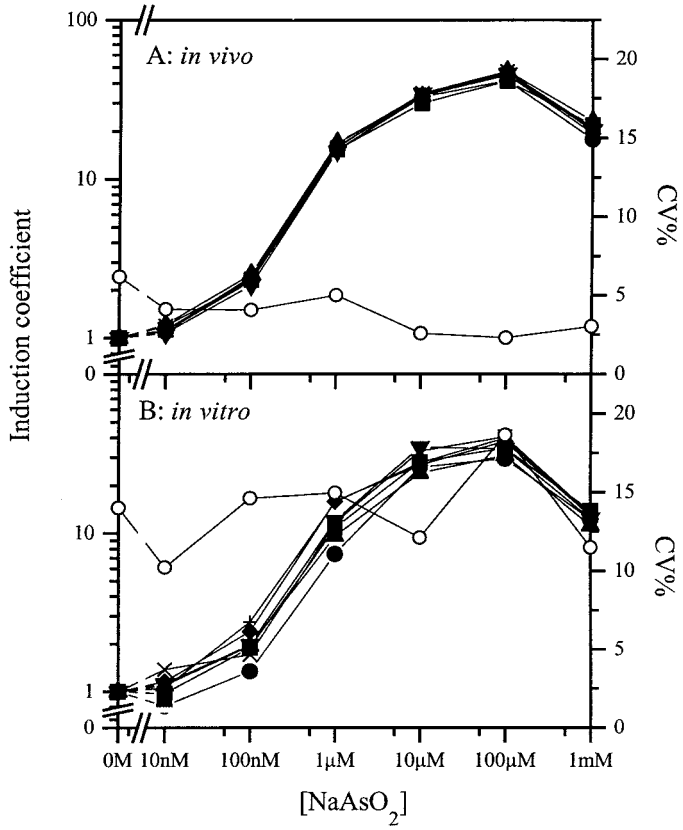

FIG. 2. Comparison of seven different water samples spiked with arsenite and measured with *E. coli* strain MC1061(pTOO31). *In vivo* measurement (A) and *in vitro* measurement (B) of spiked water samples. The coefficient of variation (CV%) of mean luminescence values between different samples (open circles) is shown at right (y axis) as percentage of luminescence value. Water samples were (solid symbols) well water (up-triangle and cross), river water (circle), brackish water (down-triangle), tap water (diamond and plus), and commercial reference water (square). Cells and different concentrations of arsenite ions diluted in different waters were incubated for 90 min and assayed for luminescence by *in vivo* and *in vitro* measurement methods. Conditions of induction and measurements are described under Materials and Methods.

DISCUSSION

The main interest of this study was comparison of two luciferase activity measurement methods: real-time analysis of gene expression from intact cells (*in vivo*) and disrupted cells (*in vitro*) using commercially

TABLE 4

Variation of Luminescence between Six Environmental Samples and Purified Water as Measured with the *in Vitro* and *in Vivo* Methods Using Arsenic Ion-Sensitive Strain *E. coli* AW3110(pTOO31)

	In vivo			*In vitro*		
[$NaAsO_2$] (M)	Mean (RLU)	SD (RLU)	CV (%)	Mean (RLU)	SD (RLU)	CV (%)
0	9.84	0.845	8.59	10.90	3.556	32.62
1×10^{-7}	12.14	1.326	10.92	11.53	3.378	29.29
1×10^{-6}	40.49	4.358	10.76	37.80	6.830	18.07
1×10^{-5}	159.82	17.611	11.02	171.50	25.174	14.68
1×10^{-4}	169.44	28.037	16.55	238.57	56.590	23.72
Mean of CVs			11.57			23.68

Note. Na–arsenite dilutions were made into different samples and incubations and luminescence measurements were done as described under Materials and Methods. Mean values are calculated from luminescence values (in relative light units, RLU) of all seven different waters at mentioned arsenite concentrations. Corresponding SDs and CVs represent variations between samples.

available reagents. To our knowledge published data from this kind of comparison do not exist. We used constitutively luciferase expressing *E. coli* strain MC1061(pCSS810) (1) and three metal-sensing strains, a mercury sensor strain *E. coli* MC1061 (pTOO11) (13) and two new arsenite sensor strains MC1061(pTOO31) and AW3110(pTOO31), constructed for this study. The second interest of this study was to characterize the performance of the new arsenic sensors.

The *in vivo* method possessed some obvious advantages over the *in vitro* method. The greater variation between replicates in using the *in vitro* protocol (Table 3) can be caused by the additional steps needed in cell disruption and the viscous nature of the lysis reagent and lysed cells. Surprisingly, the variation between different environmental samples was also greater with the *in vitro* method (Fig. 2, Table 4 and Table 5). However, it is likely that the composition of the sample has a bigger effect on luminescence in the *in vitro* method than in the *in vivo* method because the conditions probably remain more constant inside the cells under these circumstances. Our results indicate that it is not necessary to make assays *in vitro;* in fact, the *in vivo* method for *E. coli* sensor cells can give more reproducible results with variable matrix materials such as environmental samples.

We have also tested another insect luciferase, *lucGR* of *P. plagiophthalamus*, with respect to stability of the *in vitro* measurement conditions. For those cases where the *in vitro* measurement is the method of choice, firefly luciferase proved to be a better reporter because the enzyme is more stable in the lysate than the click beetle luciferase (data not shown here). For the click beetle luciferase, the *in vivo* method seemed much better, for example when different colors of light emission are used for calibrating the system against secondary matrix-associated effects such as nonspecific toxicity.

The luminescence response as a function of number of cells is directly linear over a wide range from 1×10^5 to 8×10^6 cells/assay with both measurement methods. This makes assaying of the sensor strains possible with different types of devices capable of measuring luminescence. In this study we used a microtiter plate-reading luminometer equipped with dispensing systems which allows us to measure luminescence immediately using either peak or integration mode of measurement. However, the sample transport to the assay position of a liquid scintillation counter takes some time and therefore the kinetics of luminescence must cope with the device. The decay of *in vitro* luminescence is 1.5%/min which is sufficient for a rather slow-moving sample positioning line such as those encountered in older scintillation counters or rack luminometers. On the other hand, the *in vivo* luminescence is constant for several tens of minutes from the addition of substrate (our unpublished observations) so it can be measured during an extended period of time.

The arsenic sensor strains constructed here, *E. coli* MC1061(pTOO31) and AW3110(pTOO31), proved to be better than our previously described arsenic sensor strain *Staphylococcus aureus* RN4220(pTOO21) (14), which was based on the arsenic resistance of plasmid pI258 of *S. aureus.* The earlier sensor was somewhat less sensitive toward arsenite and it could not detect arsenate at all, in contrast to the sensor described here. In addition, freeze-drying of the *S. aureus* sensor was not very successful and also the use of the human pathogenic bacterium *S. aureus* in field testing may be questionable. There were no clear differences in sensitivity of detection between *E. coli* MC1061(pTOO31) and the arsenic ion-sensitive strain (22) AW3110 (pTOO31). One of our important goals in generating

TABLE 5

Variation of Luminescence between Six Environmental Samples and Purified Water as Measured with the *in Vitro* and *in Vivo* Methods Using Strain *E. coli* MC1061(pTOO11)

	In vivo			*In vitro*		
[$HgCl_2$] (M)	Mean (RLU)	SD (RLU)	CV (%)	Mean (RLU)	SD (RLU)	CV (%)
0	10.80	1.086	10.07	5.43	0.959	17.64
1×10^{-10}	13.50	3.493	25.88	5.83	1.924	33.01
1×10^{-8}	230.98	33.558	14.53	76.72	10.104	13.17
1×10^{-7}	534.48	129.94	24.31	135.36	49.176	36.33
Mean of CVs			18.70			25.04

Note. Mercury dilutions were made into different samples and incubations and luminescence measurements were done as described under Materials and Methods. Mean values are calculated from luminescence values of all seven different waters at mentioned mercury concentrations. Corresponding SDs and CVs represent variations between samples.

specific sensors for heavy metals is to apply them for field use to be able to rapidly evaluate possible spills directly in a critical measurement place. The field use of sensor bacteria is more convenient with lyophilized bacteria that can be used as a reagent with no need of cultivation of cells before measurements. The usage of lyophilized cells also reduces day to day variation of results. Here we have shown that the *in vivo* measurement gives more accurate and reliable results than the *in vitro* method. This is especially relevant in field use where complicated extraction/measurement protocols are not possible.

The advantage of insect luciferases as reporter genes is the low background due to lack of endogenous luminescence in the cells that are commonly used. The substrate for insect luciferase, D-luciferin, penetrates membranes only in its protonated form, i.e., under acidic conditions. Usually pH 5.0 is used but there are some differences concerning the optimal pH since for example the optimal pH value for recombinant *Streptococcus mutans* is 6.0 rather than 5.0 (26). After all, the measurement of bioluminescence from whole cells can be performed *in vivo,* obtaining a measurable signal, which follows dose response with regard to analyte (inducer) concentration and cell count.

Use of GFP as a reporter gene is rapidly increasing partly due to various mutants of green fluorescent protein (10, 27) that have been introduced recently. Detection of GFP is possible without disruption of cells which allows real-time monitoring. Furthermore, GFP does not participate in any reaction in the cell since it is not an enzyme, which means that it does not disturb cell metabolism. However, it must be noted that insect luciferase participates in cell metabolism only when its substrate is externally added. Therefore, insect luciferase does not disturb the metabolism of sensor cells during the sensing reaction (induction of luciferase synthesis) as long as D-luciferin is not added, so the situation is similar with GFP. Luciferases have the advantage of being virtually free of background, whereas there is considerable background fluorescence when any type of cells are exposed to excitation signal. In addition, GFP is a very stable protein, which means that it accumulates inside the cells and thereby causes elevation of the background and makes following dynamic gene expression problematic, although that problem can be alleviated by using unstable variants of GFP (28). These facts presented above apply to both traditional monitoring of gene expression and the use of these reporter genes in sensor bacteria.

So far there has not been adequate data on how real-time analysis of gene expression in microbial cells (*in vivo*) is comparable to the results obtained by first disrupting the bacteria and then measuring luciferase activity by adding commercial measurement solution containing ATP, D-luciferin, and some cofactors that stabilize luciferase enzyme (*in vitro*). Our results clearly indicate that a good correlation exists between the two methods and therefore both assay methods can be utilized for analysis of insect luciferase reporter gene activity when *E. coli* is used as a host organism. It remains to be seen whether a similar *in vivo* approach is useful also for other types of cells once an optimal lysis reagent is applied. For mammalian cells several commercial protocols/reagents exist for quantitative cell lysis and analysis of firefly luciferase produced.

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