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Rapid Detection of Mutagens by Induction of Luciferase-Bearing Prophage in *Escherichia coli*

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Mutagenicity in bacteria is highly correlated with carcinogenicity in humans, and many industrial wastes and manufacturing sites contain mutagenic components. This has stimulated interest in rapid screening assays for genotoxic agents. We describe a rapid and sensitive bacterial test for detecting DNA-damaging agents, based on bioluminescence reporting of prophage λ induction. A promoterless cassette bearing genes encoding bacterial luciferase was transposed randomly into the λ chromosome from the suicide plasmid pUTmini-Tn*5luxAB*, and *Escherichia coli* JM101 was lysogenized with the resulting phages. In the lysogens, the luciferase genes are expressed from phage promoters, which allows rapid detection of DNA-damage-triggered prophage induction with a simple whole-cell luciferase assay. One lysogen was selected among several for its high ratio of induced to spontaneous luciferase activity upon exposure to mitomycin C. Within 4 h, the assay detects ethyl methanesulfonate, methyl methanesulfonate, and mitomycin C with sensitivity comparable to those of assays requiring more time, although not all tested mutagens were detected. The test is also automatable and generates very little waste, which makes it a potentially valuable tool for rapid screening and process monitoring.

Introduction

Mutagenicity in bacteria is highly correlated with carcinogenicity in humans (*1*, *2*), and many industrial wastes and manufacturing sites contain mutagenic components. In response to environmental concerns, many tests to detect mutagens have been developed over the past two decades (*3*, *4*). The robustness and rapid growth of bacteria have made them the organisms of choice for most short-term assays (*5*−*9*).

The most widely used short-term test for mutagenicity is the *Salmonella*/microsome assay (*6*, *10*), in which histidine-dependent auxotrophs of *Salmonella typhimurium* revert to prototrophy and form countable colonies on histidine-deficient medium. The frequency of reversion is increased by the presence of mutagens. The test has been validated for many compounds (*1*) and has served as the reference for subsequent assays.

Mutagens can also be detected by their ability to induce prophage λ in *Escherichia coli* (*11*−*14*). DNA-damaging agents trigger the SOS system, activating *recA*, leading to the cleavage of the λ repressor, and inducing the lytic cycle of λ (*15*). Phage-induction assays potentially detect a broader range of DNA-damaging agents than gene mutation assays, and the results are usually obtained faster. Furthermore, some phage-induction assays do not require survival of the tester strain (*13*, this study). In the Inductest (*11*), prophage induction is detected by overnight formation of plaques on a bacterial lawn. In the assay of Lee et al. (*13*), the genes encoding firefly luciferase were inserted at a specific site in phage λ and a lysogen was constructed. Prophage induction was detected by a luciferase assay, since the luciferase genes are expressed along with the phage genes upon induction. Although this test is less labor-intensive and faster than the Ames test, it was validated only for mitomycin C and UV light and is apparently less sensitive than other mutagenicity assays.

Bacterial luciferase has been extensively used as a reporter (*16*−*18*). It is a heterodimeric enzyme that catalyzes the flavin-mediated oxidation of a long-chain aldehyde to yield the corresponding carboxylic acid and blue-green light (*19*):

$$\mathrm{RCHO} + \mathrm{O_2} + \mathrm{FMNH_2} \xrightarrow{\text{luciferase}} \mathrm{RCOOH} + \mathrm{FMN} + \mathrm{H_2O} + h\nu \qquad (\lambda_{max} = 490\ \mathrm{nm})$$

In the presence of excess reactants (provided in vitro or by cellular metabolism), the amount of luciferase can be sensitively determined by measuring the emitted light intensity.

We have devised a sensitive assay that combines the advantages of luciferase reporters and phage-induction assays for mutagens. A promoterless-*luxAB* cassette (*20*) was inserted at different positions in bacteriophage λ*imm21* by transposition, and *E. coli* strain JM101 was lysogenized for the resulting phages. Upon induction of the prophage by mutagens, the luciferase genes are expressed along with the phage genes, which allows sensitive detection of the extent of induction with a simple whole-cell luciferase assay. Due to the random character of transposition, we obtained a library of lysogens, from which was selected the lysogen with the best signal to background ratio when exposed to mitomycin C. Initial results with known mutagens show that the test is sensitive and rapid.

Materials and Methods

Chemicals and Media. All chemicals were purchased from Sigma unless otherwise specified. Bacto tryptone and Bacto yeast extract were obtained from Difco. Streptonigrin (STN), 4-nitroquinoline 1-oxide (NQNO), benzo[*a*]pyrene (B(a)P), fluorene, ethyl methanesulfonate (EMS), and methyl methanesulfonate (MMS) were dissolved in dimethyl sulfoxide (DMSO). N^4-aminocytidine (N4AC), hydrogen per-

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oxide (H_2O_2), sodium azide (NaN_3), bis(chloromethyl) ether (BCME), formaldehyde, and mitomycin C were dissolved in sterile deionized water. Naphthalene was dissolved in ethanol. Solid and liquid Luria-Bertani (LB) media were supplemented, when required, with ampicillin (LB/Amp; 100 μg/mL; Boehringer-Mannheim) and tetracycline (LB/Tet; 10 μg/mL; Boehringer-Mannheim). Deionized water (Millipore) was sterilized for use as water reagent.

Equipment. Cell growth was measured at 600 nm using a Beckman DU-64 spectrophotometer and 1-cm path cuvettes; absorbances higher than 1.00 were confirmed by dilution in LB. Growth in liquid LB medium was carried out at 37 °C with agitation (300 rpm) in 25 × 150 mm glass tubes (Kimax) in a New Brunswick Scientific Gyrotory water bath shaker G76D or in 125-mL glass flasks in a Lab-Line Environ-Shaker. Growth on solid LB medium or in 24-well tissue culture plates (Falcon) with agitation (220 rpm) was performed in a NuAire NU-1700 incubator at 37 °C.

Strains and Plasmids. All bacterial strains used in this study are *E. coli* K12 derivatives. *E. coli* strain JM101 was used to propagate the bacteriophage $\lambda imm21$ and as host for the prophage. *E. coli* AB1157 and JC11801 were obtained from the *E. coli* Genetic Stock Center (Yale University, New Haven, CT) and were used as hosts for the prophage. JC11801 is isogenic to AB1157 except for an *rfa* mutation in the chromosome, which enhances its permeability. *E. coli* strain CC118(λpir) was used to propagate the π−protein-dependent plasmid pUTmini-Tn*5luxAB*, which confers a double antibiotic resistance on its host: the ampicillin resistance provided by the pUT derivative plasmid (*21*) and the tetracycline resistance of the minitransposon mini-Tn*5luxAB* (*20*).

Transposition of Mini-Tn*5luxAB* into the Phage Chromosome. Plasmid pUTmini-Tn*5luxAB* was transformed into CC118(λpir) by electroporation (Electroporator II; Invitrogen) under the following conditions: capacitance 50 μF, resistance 150 Ω, and voltage 15 kV/cm. Transformants were selected for ampicillin and tetracycline resistance. An aliquot (100 μL; ca. 10^8 cells) of an overnight culture of donor strain CC118(λpir) harboring the pUT derivative minitransposon plasmid was infected with 100 μL of a $\lambda imm21$ plaque suspension (10^5 phages) in a culture tube. After phage adsorption at 37 °C for 20 min, 2 mL of LB was added, and the infected culture was allowed to grow for 6 h. Cell lysis was completed by addition of 40 μL of chloroform and additional shaking for 15 min. The supernatant was harvested by centrifugation at 14 000 rpm for 10 min and sterilized with chloroform, and the resulting phage lysate was stored at 4 °C.

Construction of the Lysogens. A fraction (500 μL, ca. 5×10^9 pfu) of the phage lysate obtained from CC118-(λpir)+pUTmini-Tn*5luxAB* infection by $\lambda imm21$ was mixed with 1.3 mL of an overnight culture of JM101 (6.2×10^9 cells). After adsorption at 37 °C for 20 min, 2 mL of LB was added and the mixture was further grown for 45 min. The culture was centrifuged at 10 000 rpm for 2 min, and the pellet was resuspended in 250 μL of LB and plated on LB/Tet medium. Eight of the resulting tetracycline-resistant colonies were tested for luciferase background activity and luciferase-induced activity by mitomycin C. Each colony was grown in LB/Tet medium to mid-log phase; each culture was then divided in two equal aliquots, and mitomycin C was added to a final concentration of 1 μg/mL to one aliquot; each aliquot (uninduced and induced) was further grown for 1.5 h before being assayed for luciferase activity from 200 μL of culture. The strain with the lowest background activity (signal to background ratio equal to 2.1 after this short exposure) was designated JM101(λlux4).

A 1.5-mL overnight culture of JM101(λlux4) was induced by mitomycin at 1 μg/mL for 3 h. The culture was centrifuged at 10 000 rpm for 5 min. A fraction of the phage supernatant (300 μL) was mixed with 1 mL of an overnight culture of AB1157 or JC11801. After incubation at 37 °C for 20 min, 3 mL of LB was added, and the infected cells were further grown for 45 min. The cultures were centrifuged at 10 000 rpm for 2 min and the pellets resuspended in 250 μL of LB and plated on LB/Tet medium. The resulting lysogens were designed AB1157(λlux4) and JC11801(λlux4).

Whole-Cell Luciferase Assay. Luciferase activity was measured using a luminometer (Model 20e, Turner Designs) adjusted to a sensitivity of 2.1×10^6 quanta-s^{-1} (light unit)$^{-1}$, referenced to the radiochemical standard of Hastings and Weber (*22*). The substrate used for the luciferase assay was a 5% v/v solution of n-decanal in ethanol. The assay was performed by mixing 1 mL of cell culture with 10 μL of freshly prepared substrate solution in a 20-mL scintillation vial (Wheaton), swirling for 15 s, and integrating the time course of light emission for 10 s. When the luciferase activity of 1-mL aliquots exceeded the range of the luminometer, the assay was repeated with a 200−μL aliquot, and values obtained in this way were rescaled to 1 mL when presented below. Control experiments showed that this scale-up procedure altered the results by only 20%, while the luminometer reproducibility is ±10%. In the present work, the 5% v/v solution of decanal in ethanol was prepared in glass vials immediately before the luciferase assay was performed. Sinclair et al. (*23*) reported that the age of the decanal in ethanol solution used as substrate for the luciferase assay can influence the results. We confirmed this observation and also found that decanal preparations were more stable over time in glass vials than in polypropylene tubes: the signal for the same culture was 1.4 times (for glass vials) and 1.5 times (for polypropylene tubes) higher for 2-h-old decanal solutions than for fresh solutions.

Exposure to Mutagens. An overnight culture of JM101-(λlux4) in LB/Tet at 37 °C was diluted 1:400 into fresh LB/Tet medium. The culture was grown to $A_{600} = 0.3-0.7$. In a typical dose−response experiment, culture fractions (1.25 mL) were distributed into wells of γ-sterilized polystyrene 24-well tissue culture plates containing 10 μL of the appropriate dilution of the compound to be tested. Each experiment was done in quadruplicate, and negative solvent controls were included. Plates were incubated at 37 °C with agitation (220 rpm) on a Lab-Line Orbit Shaker for 3.5 h. A luciferase assay was then performed for each well. The mutagenic activity of a compound at a certain concentration was expressed by the induction factor, which is the ratio of the luciferase activity in the presence of the compound to its value in the absence of test compound (background) at the same time.

Rat Liver Extract S9. Rat liver extract S9 from Aroclor 1254-induced animals was purchased from Microbiological Associates (Bethesda, MA). The cofactor mix contained 60 mM KCl, 10 mM $MgCl_2$, 8 mM glucose 6-phosphate, 5 mM NADP in 0.18 M sodium phosphate buffer (pH 7.4), and 9.8% v/v S9. The final concentration of S9 in the assay mixture was 9 μL/mL.

Disposal of Mutagens. Contaminated disposables were placed in biohazard heavy-duty plastic bags. The bags were sealed and placed in cardboard boxes. Liquid waste was

stored in glass bottles. The boxes and bottles were sealed and labeled with the contents before disposal by the Environmental and Physical Safety Department of the University of Houston.

Results and Discussion

Construction and Stability of the Lysogens. After lysogenization of JM101 with the phage lysate, tetracycline-resistant colonies were obtained. The cognate transposase gene was not carried with the minitransposon in *λimm21* (*24*); therefore, colonies resistant to tetracycline were expected to be lysogens rather than new transposition events. This was verified by harvesting the supernatants of cultures from individual colonies and spotting these supernatants on a JM101 lawn; a clear lytic zone was visible at all supernatant spots.

Eight candidate lysogens were screened for their responsiveness to brief mitomycin C induction. The luciferase activities for the uninduced cultures ranged from 8.22 light units (LU) to 8425 LU [mean 2922 LU; standard deviation (SD) 3728 LU]; the activities for the induced cultures ranged from 16.69 to 7980 LU (mean 2620 LU; SD 3197 LU); and the induction factors varied from 0.78 to 2.50 (mean 1.37; SD 0.67). Since the *luxAB* genes are promoterless in the minitransposon (*20*), the background activity is likely due to spontaneous induction of the prophage or to insertion of *luxAB* genes downstream from a constitutively expressed phage promoter. The lysogen exhibiting the lowest background luciferase activity (8.22 LU; induction factor 2.1 under these conditions) was designed as JM101(λlux4) and used for optimization of the assay.

The stability of the minitransposon insertion was verified by growing JM101(λlux4) in LB without antibiotics for 40 generations and plating dilutions of the final culture on LB and LB/Tet plates; the number of colonies on the LB/Tet and on the LB plates was the same.

The other phage/luciferase-based assay for mutagens involved cloning of the luciferase genes into a specific site in phage λ (*13*). Although this approach allows a better characterization of the lysogen, our strategy allowed selection of the lysogen with the best characteristics from among many, leading to better sensitivity.

Influence of Solvents. We used H_2O, ethanol, and DMSO as solvents for the mutagens. As expected, H_2O added alone in small quantities had no effect on the signal. Since we were adding the same volume of the appropriate mutagen dilutions to each well, the concentration of DMSO differed from well to well (between 0.0 and 0.8% v/v DMSO in the final culture). The maximal DMSO concentration (0.8% v/v) induced a marginally detectable increase of the luciferase activity over its value in the absence of DMSO (ratio = 1.6). However, in all experiments, the luciferase activity in the presence of mutagen was normalized by its value in the presence of the highest solvent concentration (0.8% v/v), resulting in a conservative reporting of the assay sensitivity.

Older bottles of DMSO have been reported to to accumulate phage-inducing material (*14*), and we have investigated the possibility of using other solvents. While ethanol, toluene, and chloroform exhibited a slight toxicity indicated by a decreasing luciferase activity with increasing solvent concentration (induction ratios at 0.8% v/v solvent were 0.7 ± 0.2 for ethanol, 0.6 ± 0.1 for toluene, and 0.4 ± 0.3 for chloroform), they did not cause prophage induction.

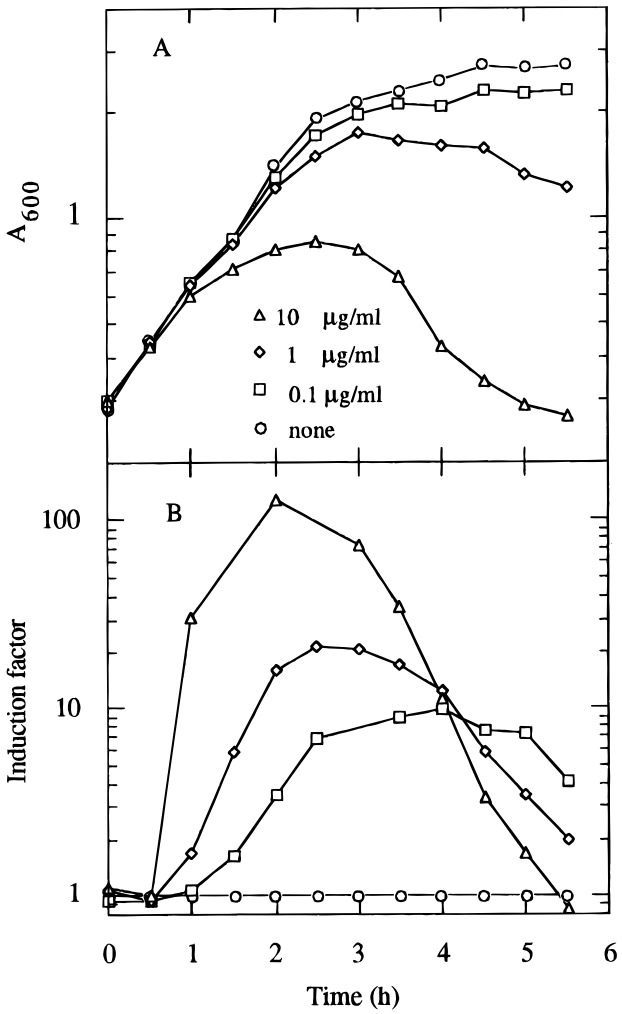

FIGURE 1. Kinetics of induction of JM101(λlux4) by several concentrations of mitomycin C. An overnight culture of the lysogen in LB/Tet was diluted 1:100 into fresh LB/Tet medium. The culture was grown to $A_{600} \approx 0.3$, aliquoted in 125-mL flasks (50 mL in each), and several concentrations of mitomycin C were added at time zero, as indicated on the graph; one culture (○) was not induced. All subcultures were grown at 37 °C, 300 rpm. The absorbance at 600 nm (A) and the luciferase activity (B) were monitored every 30 min. Due to the finite dynamic range of the luminometer, the ordinate values at 1.5 h and 2.5 h for the 10 μg/mL curve and at 3 h for the 0.1 μg/mL curve are lower bounds.

Kinetics of Induction of JM101(λlux4) by Mitomycin C. The kinetics of luciferase induction and inhibition of growth of JM101(λlux4) by several concentrations of mitomycin C was studied (Figure 1). As expected, the higher the mutagen concentration, the more observable its cytotoxicity, as shown by the decrease of absorbance at higher mutagen concentrations.

The induction factor increased observably 30−60 min after induction, which is consistent with the fact that the λ repressor activity disappears within 30 min of induction and that phage replication is occuring during this time (*25*). This rapid response allows detection of mutagens within 1 h if speed is more important than optimal sensitivity.

The luminescence curve had the same dome shape at all concentrations, rising up to a maximum before decreasing toward zero. The incubation time giving the maximal induction factor was longer for low mitomycin C concentrations (4 h for 0.1 μg/mL mitomycin C; 2.5 h for 1 μg/mL; and 2 h for 10 μg/mL). As a result, when testing

TABLE 1

Maximal Induction Factor Obtained for Several Mutagens with JM101(λlux4)[a]

compd	concn tested (μg/mL)	max induction factor	concn giving max induction factor (μg/mL)	detection limit[b] (μg/mL)
H_2O_2	0−500	31.5 ± 5.5	50	5
mitomycin C	0−2	26.9 ± 2.8	0.5	0.005
streptonigrin	0−2	19.8 ± 4.3	0.5	0.0005
MMS	0−3000	12.8 ± 0.6	500	10
fluorene	0−2400	6.9 ± 1.0	2400	0.4
EMS	0−8000	6.1 ± 0.7	4000	50
NQNO	0−2	3.3 ± 0.0	1	0.25
N4AC	0−80	2.6 ± 0.7	80	5
formaldehyde	0−300	2.3 ± 0.2	30	30

[a] The concentrations are the concentrations of mutagen in the assay mixture. The experiments were performed as explained in Materials and Methods. [b] Lowest concentration giving light levels greater than two standard deviations above the mean light level in absence of compound.

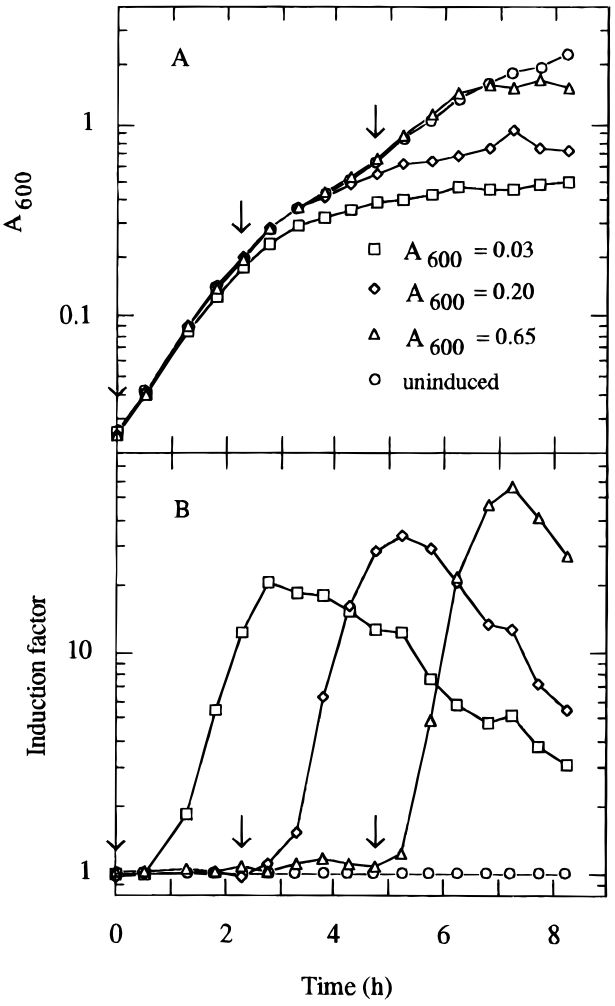

FIGURE 2. Influence of cell density at time of induction on the absorbance (A) and the luciferase activity (B). An overnight culture of JM101(λlux4) in LB/Tet was diluted 1:100 into fresh LB/Tet medium. The culture was grown for 1 h, aliquoted in five 125-mL flasks (50 mL in each), and the resulting subcultures were grown at 37 °C, 300 rpm until induction. At different times (indicated by arrows) corresponding to the specified cell densities, mitomycin C was added to one flask at a final concentration of 1 μg/mL. One culture was not induced (○) and was used as reference (background). The luminescence of the reference culture increased from 0.68 up to 550 LU after 8.25 h because of growth.

for low concentrations of a compound in a dose−response study, the luciferase activity should be assayed well after induction, but not so late that mutagenic activity is hidden by toxicity. Continuous activity monitoring, which is feasible with luciferase, may be useful for optimal sensitivity.

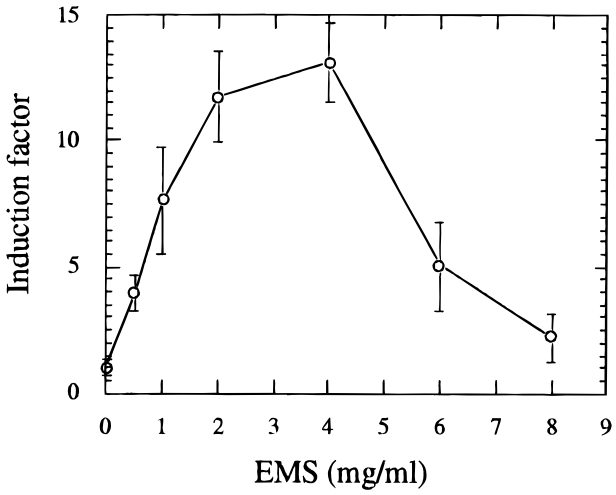

FIGURE 3. Dose−response curves for the alkylating agent EMS. The absorbance of the culture at the time of induction was 0.30 and 0.51. The induction factor is plotted versus the concentration of mutagen in the culture. The error bars are the mean ± SD from four experiments. The background activity was 108.5 ± 0.4 LU.

The incubation time for dose−response studies was chosen to be 3.5 h.

Influence of the Cell Density at Time of Induction. We studied the effect of culture density on the induction factor at the time of induction by 0.1 μg/mL mitomycin C (Figure 2). The incubation time required to reach the maximal induction factor was slightly shorter for denser cultures (2.5 h for a culture induced at $A_{600} = 0.65$ vs 3.25 h for one induced at $A_{600} = 0.03$), and this maximum was also higher. This is consistent with the fact that, at high cell density, more targets are available for induction by mutagens. To maximize sensitivity, therefore, the cultures were subsequently induced in late exponential phase for dose−response studies.

Response to Standard Mutagens. Several known mutagens induced luciferase expression from JM101(λlux4) (Figure 3 and Table 1). In nearly every case, the induction factor was observed to decline at the highest concentrations tested, presumably because of toxicity.

Sensitivity of the Assay. The sensitivity of the assay can be compared with those of previously described mutagenicity tests on the basis of amount of mutagen per assay (as reported in Table 2), the concentration of mutagen in the sample, or the concentration of mutagen in the assay mixture. We report here the comparison based on amounts of mutagen detected because of the lack of published concentration information for the assay of Lee et al. (*13*).

TABLE 2

Comparative Sensitivities of Bacterial Mutagenicity Tests

compd	present test[b]	Lee et al. (*13*)	Inductest[c]	SOS Chromotest	Ames test
mitomycin C	6	100	10	0.5	<500*
EMS	63 000	–	neg (?)	61 000	3 000 000
MMS	13 000	–	neg (?)	2000	70 000
NQNO	313	–	9.5	1.1	9.5
B(a)P	neg (62 500)	–	100	180	1000
streptonigrin	0.6	–	–	–	<100*
H_2O_2	6000	–	–	–	$<10^{6}$*
fluorene	500	–	–	–	neg (10^{9})
naphthalene	neg (1.25×10^{6})	–	–	–	neg (10^{8})
formaldehyde	38 000	–	–	–	$<10^{6}$*
NaN_3	neg (13 000)	neg (?)	–	–	–
BCME	neg (10^{6})	–	–	–	–
N4AC	6000	–	–	–	–

[a] Data for the Inductest, the SOS Chromotest, and the Ames test were obtained from ref 26 (or from ref 29, indicated by *). (neg) only background activity for concentrations up to that indicated in parentheses; (?) no concentration was reported in the literature; –, not tested. [b] The sensitivity is defined as the lowest quantity (in nanograms per assay) giving light levels greater than two standard deviations above the mean light level in the absence of compound. [c] The sensitivity is defined as the lowest quantity (in nanograms per assay) giving light levels twice the mean light level in the absence of compound (induction factor ≥2).

The fact that the present test detects the alkylating agents EMS and MMS, while the mechanistically similar Inductest was not reported to do so (*26*), is probably due to the high sensitivity with which the luciferase reporter activity can be measured. The detection of lower mitomycin concentrations than in Lee's test may arise in part from the more rapid transport of aldehydes through cell membranes with respect to luciferin (*27*). The bulky quinone streptonigrin, the base analog N4AC, formaldehyde, and the oxidizing agent H_2O_2 also induced the prophage, but comparable literature data could not be found for these compounds. The assay also detected fluorene, which was reported as nonmutagenic in the Ames test (*26*). The assay detected NQNO, but with a lower sensitivity than the other assays. Sodium azide did not induce the prophage up to a concentration of 10 μg/mL; sodium azide is mutagenic in the *Salmonella*/microsome assay (*6*) but is not reported to be carcinogenic (*28*). Other compounds that did not induce the prophage include B(a)P, the alkylating agent BCME, and naphthalene.

In practical terms, the sensitivity is best expressed as the lowest detectable mutagen concentration in the sample. We investigated the possibility of improving the sensitivity of the assay to dilute mutagens in two experiments. In the first experiment, a constant quantity of mitomycin C dissolved in different volumes was added to volumes of JM101(λlux4) culture sufficient to make a final volume of 50 mL (the final mitomycin C concentrations were therefore equal). The maximal induction factor when the mutagen was added as a 160-fold diluted solution was only 50% below that obtained with the concentrated mutagen solution. In the second experiment, increasing volumes of the same mitomycin C solution were added to a constant cell culture volume (Figure 4). The luminescence increased with added volumes up to 25 mL and then decreased rapidly. These results suggest that the analysis of dilute aqueous streams may be enhanced by testing larger sample volumes.

Influence of Rat Liver Extract S9 and Strain Permeability. Some chemicals, such as benzo[*a*]pyrene, require metabolic activation. To determine the influence of the *rfa* mutation and of S9 activation on lysogen induction, we studied the time course of induction of AB1157(λlux4) and its *rfa* derivative JC11801(λlux4) by 9 μg/mL B(a)P in the presence or absence of 9 μL/mL S9. Neither the addition of S9 nor the introduction of the *rfa* mutation significantly increased the B(a)P-induced signal over the base case (AB1157 without S9).

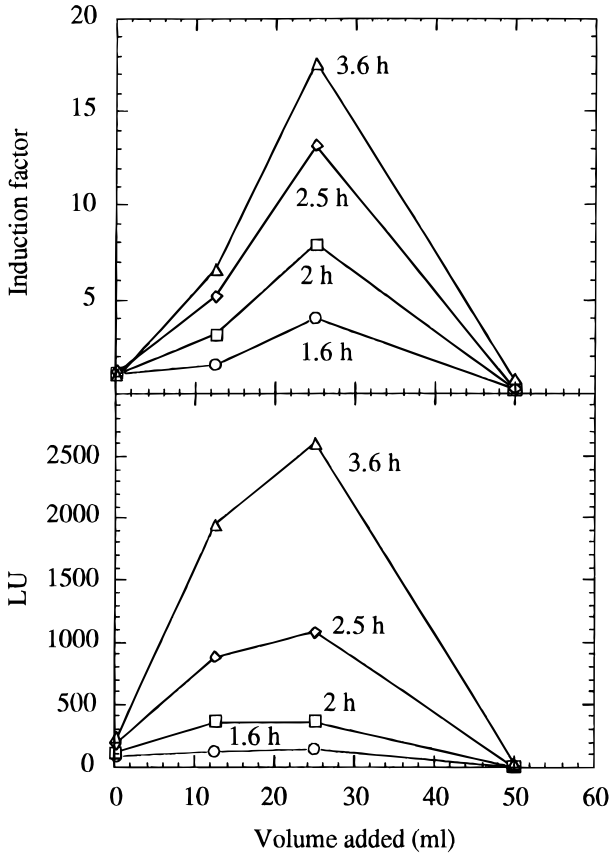

FIGURE 4. Influence of the added sample volume on the induction of JM101(λlux4). An overnight culture of JM101(λlux4) in LB was diluted 1:100 into fresh LB medium. The culture was grown to $A_{600} \approx 0.8$ and distributed into eight 125-mL flasks (25 mL in each). Different volumes of a 15 ng/mL mitomycin C in 10 g/L NaCl solution were added to four flasks. The same volumes of 10 g/L NaCl solution were added to the other four flasks as reference cultures. All eight flasks were grown aerobically (300 rpm) at 37 °C. The luciferase activity (LU) and the induction factor are reported as a function of the volume added for several incubation times.

Recommended Testing Procedure for Water Samples. Any sample to be tested for the presence of mutagens should

be serially diluted before the assay to avoid false negative results due to toxicity at high concentration; 5-fold dilutions cover a useful range of concentrations. A late log-phase culture of JM101(λlux4) in LB/Tet (A_{600} = 0.7−1.0) is distributed (900−μL aliquots) into wells of polystyrene 24-well tissue culture plates containing 600 μL of the appropriate dilution of the compound to be tested. A negative control consisting of 900 μL of culture to which 600 μL of deionized water is added should be included. A positive control consisting of 900 μL of culture to which 600 μL of 1.25 μg/mL mitomycin C solution is added should also be included. Plates are incubated at 37 °C with agitation (220 rpm) for 3.5 h. A luciferase assay is then performed on 1 mL of culture for each well.

In conclusion, the assay is easy to perform, as it is based on a single strain and the simple, rapid, and sensitive whole-cell assay of luciferase activity. It detects a variety of classes of mutagens (alkylating agents, base analogs, DNA cross-linking agents, and oxidizing agents) and is rapid, giving a response within 1−4 h. It is inexpensive, generates less waste than the currently available tests, and may prove useful for routine monitoring of some mutagens in environmental and process samples.

Acknowledgments

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