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A Biosensor for Environmental Genotoxin Screening Based on an SOS *lux* Assay in Recombinant *Escherichia coli* Cells

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A genetically controlled luminescent bacterial reporter assay, the SOS *lux* test, was developed for rapid detection of environmental genotoxins. The bioassay is based on the recombinant plasmid pPLS-1, which was constructed as a derivative of pBR322, carrying the promoterless *luxCDABFE* genes of *Photobacterium leiognathi* downstream of a truncated *cda* gene from ColD with a strong SOS promoter. *E. coli recA*$^+$ strains containing this construction are inducible to high levels of light production in the presence of substances or agents that cause damage to the DNA of the cells. The light signal, reflecting the SOS-inducing potency, is recorded from the growing culture within 1 s, and the test results are available within 1 to 2 h. Induction of bioluminescence was demonstrated by treatment of *E. coli* C600(pPLS-1) with 6 genotoxic chemicals (mitomycin C, *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine, nalidixic acid, dimethylsulfate, hydrogen peroxide, and formaldehyde) and with UV and γ radiation. A clear dose-response relationship was established for all eight genotoxins. The sensitivity of the SOS *lux* test is similar to that of other bioassays for genotoxicity or mutagenicity, such as the SOS chromotest, *umu* test, and Ames mutatest. These results indicate that the SOS *lux* test is potentially useful for the in situ and continuous detection of genotoxins.

Increasing levels of environmental pollution demand specific and sensitive detection methods for environmental toxins. To assess the impact of toxic agents on public health and ecosystem balance, biological test systems have been developed and applied to complement chemical and physical detection methods (33). Bioassays for genotoxicity assessment are based on the response to DNA damage induced by the genotoxin in the cells. The results frequently are used to infer the mutagenic and carcinogenic hazard posed to humans and other biota. The Ames mutatest uses a set of strains of *Salmonella typhimurium* that revert to histidine prototrophy upon exposure to mutagens of specific mechanisms (1, 2). Examples are 9-aminoacridine or diethylsulfate, causing frameshift mutations (15), and *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine (MNNG), sodium azide, or methylmethanesulfonate, causing base pair substitutions (18), as well as oxidative compounds or ionizing radiation (12, 16). The Ames mutatest was validated in studies using several hundred chemicals (reviewed in reference 20). Nearly 90% of the tested mutagens are also carcinogenic.

SOS-dependent bacterial test systems make use of the fact that in response to DNA-damaging agents, a cascade of functions known as the SOS response is induced. This includes the synthesis of a number of proteins involved in mutagenesis, such as RecA and UmuCD (31, 38). In the SOS chromotest, *Escherichia coli* PQ37 cells with the structural gene for β-galactosidase, *lacZ*, under the control of an SOS-controlled gene, *sulA*, are used as a test system for genotoxicity (11, 13, 27, 29). The *umu* test makes use of a recombinant *S. typhimurium* TA 1535(pSK1002) strain with a fused *umuC*::*lacZ* operon carried on a plasmid (22–24). In both systems, the SOS induction potency is determined from a colorimetric assay for β-galactosidase synthesized in response to a genotoxin. The lower limits of detection of genotoxins are in the nanomolar to micromolar range, depending on the genotoxin under investigation (19, 30). There is a close correlation between the results of the Ames mutatest and SOS tests: among several hundred chemical compounds tested, 82% give similar responses in Ames mutatest and SOS chromotest (30). Sixty to seventy percent of the genotoxins that induce the SOS response are carcinogenic to mammals (30). Although the specificity for carcinogens of the SOS test is lower than that of the Ames mutatest, the number of false-positive compounds is lower in the SOS test. Therefore, the results of both tests can complement each other.

Recently, genetically controlled bacterial luminescent biosensors have been developed to detect a variety of environmental pollutants (reviewed in references 5 and 35). They are characterized by the coupling of a receptor component (e.g., promoters that are subject to environmental regulation) with a reporter component (e.g., the genes required for bioluminescence). Several recombinant bacteria with plasmids carrying the promoterless *lux* operon (*luxCDABE*) originating from *Vibrio fischeri* or other marine bacteria—or only the *luxAB* genes coding for the bacterial luciferase subunits α and β—under control of a special inducible promoter have been developed for, e.g., the detection of mercury (34, 36), arsenic and cadmium (6), or naphthalene and salicylate (4, 8). These biosensors excel in a rapid induction of bioluminescence within 1 h in response to the applicable environmental pollutant.

We have constructed a biosensor with specificity for genotoxins by combining the SOS system, indicative of DNA-dam-

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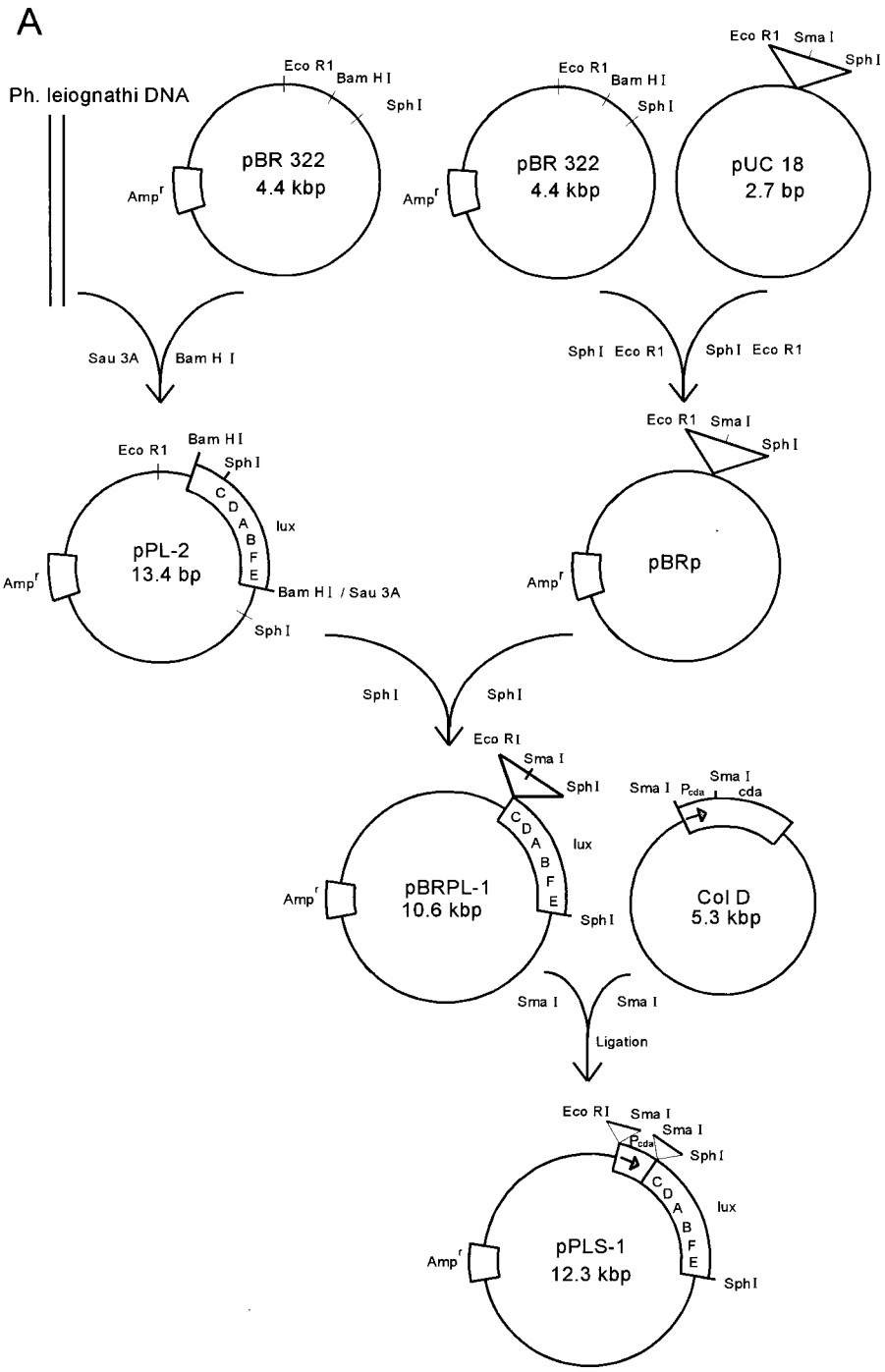

aging agents, as a receptor component with the bioluminescence system as a rapid reporter component. Recombinant *E. coli* C600(pPLS-1) carries a plasmid with the promoterless *lux* operon (*luxCDABFE*) of *Photobacterium leiognathi* under control of a strong SOS promoter that originates from part of the *cda* gene of the plasmid ColD (7). This SOS *lux* test responds sensitively to a variety of genotoxins, such as mitomycin C (MMC), MNNG, nalidixic acid (NA), dimethylsulfate (DMS), hydrogen peroxide (H_2O_2), formaldehyde (CH_2O), and UV and γ radiation. It allows the detection of *lux* gene expression within 1 to 2 h without disruption of the bacterial cells. The sensitivity of the SOS *lux* test is comparable to that of other known assay systems, such as the Ames mutatest, the SOS chromotest, and the *umu* test, for mutagens or genotoxins.

MATERIALS AND METHODS

Molecular cloning techniques and construction of the pPLS-1 plasmid. DNA manipulations, including plasmid isolation and molecular cloning, were performed by standard methods, as described previously (17). DNA sequencing was performed according to the method of Sanger et al. (32) by use of T7 DNA

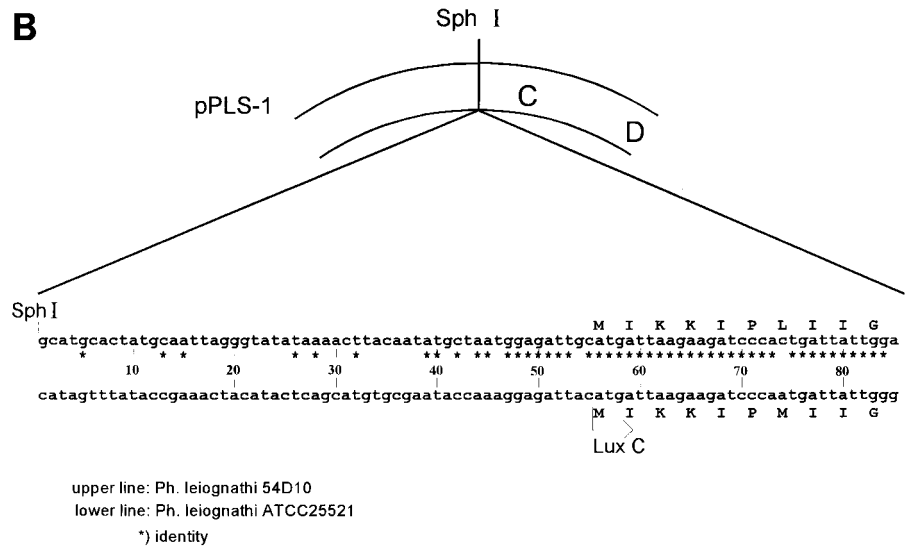

FIG. 1. (A) Construction of the bioluminescent reporter plasmid pPLS-1. The polylinker regions from pUC18 are shown magnified as triangles, and the arrows represent the SOS promoter of the *cda* gene of ColD; only those restriction sites which were used in the cloning procedure are shown. (B) Sequence of the 5′ end of the *P. leiognathi* 54D10 *lux* operon of pBRPL-1, as determined after subcloning of the *lux* operon into pUC18 and comparison with that of *P. leiognathi* ATCC 25521 (14).

polymerase. The following plasmids were used for cloning: pBR322 (Pharmacia), pUC18 (Pharmacia), and ColD (7).

Figure 1A shows the scheme of construction of plasmid pPLS-1, which carries the promoterless *lux* (*luxCDABFE*) operon of *P. leiognathi* under control of a strong SOS promoter. The *lux* operon of *P. leiognathi* 54D10 (Krasnojarsk Institute of Biophysics Collection), as a partially *Sau*3A-restricted 9-kbp fragment of its DNA, was first cloned into the *Bam*HI site of plasmid pBR322 as described previously (25). The resulting plasmid, pPL-2, carries a 9-kbp fragment with the complete *lux* operon providing full autoregulated expression of bioluminescence in *E. coli* cells (25).

To obtain a promoterless *lux* operon, the following sequential steps were taken: (i) the *Eco*RI-*Sph*I fragment of pBR322 was replaced by the *Eco*RI-*Sph*I polylinker fragment of pUC18 in order to remove the *tet* promoter from pBR322 and add suitable sites for cloning; (ii) the 6.7-kbp *Sph*I-*Sph*I fragment of plasmid pPL-2 carrying the *lux*CDABFE genes but not the *lux* promoter was inserted into this new plasmid at the *Sph*I site located at the end of the polylinker fragment. The resulting plasmid, pBRPL-1, transformed into *E. coli* C600 cells does not express bioluminescence (26). The absence of bioluminescence suggests a deletion of the *lux* promoter during the last cloning step. To obtain further support for this supposition, we used the sequence data of the bioluminescence operon of *P. leiognathi* from ponyfish (ATCC 25521) (14) to localize the starting codon of the *luxC* gene. As shown in Fig. 1B, the ATG codon, starting the translation of the *luxC* gene, is located 56 bp downstream of the *Sph*I site. The untranslated sequence of the *luxC* gene deviates from that of *P. leiognathi* ATCC 25521 in several regions (Fig. 1B), and complete promoter-like sequences are missing. Although the hexamer located between bases 33 and 39 closely resembles an *E. coli* −10 canonical promoter (5 of 6 bases match), it is localized too tightly to the ribosome binding site of the *luxC* gene and lacks the suitable structure of the −35 region of the *E. coli* promoter. Therefore, a promoter function cannot be executed.

As an SOS-dependent promoter for the *lux* operon, we chose the *Sma*I-*Sma*I fragment of ColD (7) carrying a truncated *cda* gene with a strong SOS promoter. This *Sma*I-*Sma*I fragment of ColD contains a 1.7-kbp fragment of the coding region of the *cda* gene (less than half of the gene). It was inserted into pBRPL-1 at the *Sma*I site of the polylinker fragment. The resulting plasmid, designated pPLS-1 (DSM 10333) (Fig. 1A) carries the *luxCDABFE* genes downstream of the SOS promoter and thereby has all functions necessary for SOS-inducible bioluminescence. pPLS-1 was used to transform *E. coli* C600 cells. Recombinant *E. coli* C600(pPLS-1) was stable over long periods of incubation and was used as the reporter strain in the SOS-dependent *lux* test.

Bacterial strains and growth conditions. *E. coli* K-12 C600 (*F*⁻ *thi-1 thr-1 leu136 lacYI tonA21 supE44*) (3) was used as the host in all studies. Recombinant *E. coli* C600(pPLS-1) cells were grown overnight at 37°C in L medium (21) supplemented with 50 μg of ampicillin per ml for positive selection of cells carrying plasmid pPLS-1. After dilution (1:50) in fresh L medium, the culture was incubated at 37°C until the optical density at 560 nm (OD_{560}) reached 0.2 to 0.3. Aliquots of this culture were used for the genotoxicity assay.

SOS *lux* assay for chemical genotoxins. The following chemicals and concentrations were used in the assay: 10 nM to 1 μM MMC (P-L Biochemicals), 0.5 μM to 0.1 mM MNNG (Sigma), 5 μM to 0.1 mM NA (Boehringer, Mannheim, Germany), 5 μM to 0.5 mM DMS (Sigma), 30 μM to 15 mM H_2O_2 (Reachim), and 0.15 to 1.5 mM CH_2O (Sigma). In addition, acetone (AC) (Reachim), *n*-butanol (BUT) (Reachim), phenol (PHE) (Reachim), tetracycline hydrochloride (TEC) (Serva), and chloramphenicol (CAM) (Serva) were used as negative controls. Aliquots (0.18 ml) of the test culture were transferred to polypropylene luminometric tubes, and 20 μl of appropriate concentrations of the test chemical in aqueous solution was added. Twenty microliters of distilled water was added to the control tubes. After incubation with aeration at 37°C for 90 min, and then at room temperature for 10 min, the light emission (in millivolts) was measured (LKB Luminometer 1250; Pharmacia Biotech, Uppsala, Sweden) and simultaneously the cell density (OD_{560}) was determined by use of a spectrophotometer. Light measurements were done within 1 s. Experiments were performed three times, and the standard errors between the experiments were determined.

SOS *lux* assay for physical genotoxins. UV radiation at 254 nm (low pressure Hg lamp) at a dose rate of 0.1 W/m² and γ rays (^{137}Cs source, Svet) at a dose rate of 0.4 Gy/s were used in this assay. For γ irradiation, aliquots of the cultures were irradiated on ice; for UV irradiation the cells were first harvested by centrifugation and then resuspended in M9 buffer (21) to prevent absorption of UV radiation by the medium. Aliquots of 1 ml of the irradiated suspension were transferred into 25 ml of L medium and incubated at 30°C with shaking for 2 h. Then, 0.5 ml was transferred into luminometric tubes, and light emission (MIPHI luminometer; Moscow Engineer Physics Institute) and cell density (OD_{560}) were measured. Light measurements (U/s) were carried out at 30°C for 1 s. To prevent photoreactivation, all UV experiments were done under yellow light.

Numerical analysis. The factor of SOS induction, F_i, is equal to the relative light emission, Lux_i/Lux_0, divided by the relative OD_{560} values, OD_x/OD_0: $F_i = (Lux_i \times OD_0)/(Lux_0 \times OD_x)$, where Lux_i is the light emission of the culture treated with the genotoxin (in millivolts or U/s), Lux_0 is the light emission of the culture without genotoxin, OD_0 is the optical density of the untreated culture, and OD_x is the optical density of the treated sample. This correction for cell concentration is necessary, because some genotoxins delay or impair cell growth and thereby influence the total light emission of the culture. For three chemicals, the relative optical density was compared with the survival of the cells, as determined by colony counting. All experiments were performed at least three times. Mean values and standard errors of F_i were determined for each dose. F_i was plotted as a function of the dose of the genotoxin.

Different criteria have been used in SOS tests to evaluate the degree of genotoxicity (30). We have derived two measures of genotoxicity from the dose-response curves (for this purpose we used the linear plot of F_i versus dose): (i) the lower limit of detection, which is the dose at which the induction factor F_i reaches a value twice that of the background (20), and (ii) the genotoxic potential, which is the slope of the response curves at low doses. A substance is identified to be genotoxic if at any of its concentrations F_i reaches a value of 2 or more.

RESULTS

Kinetics of SOS induction. Following damage to the DNA of the *E. coli* C600(pPLS-1) assay system by treatment with a genotoxin, the SOS response, which includes a diverse set of

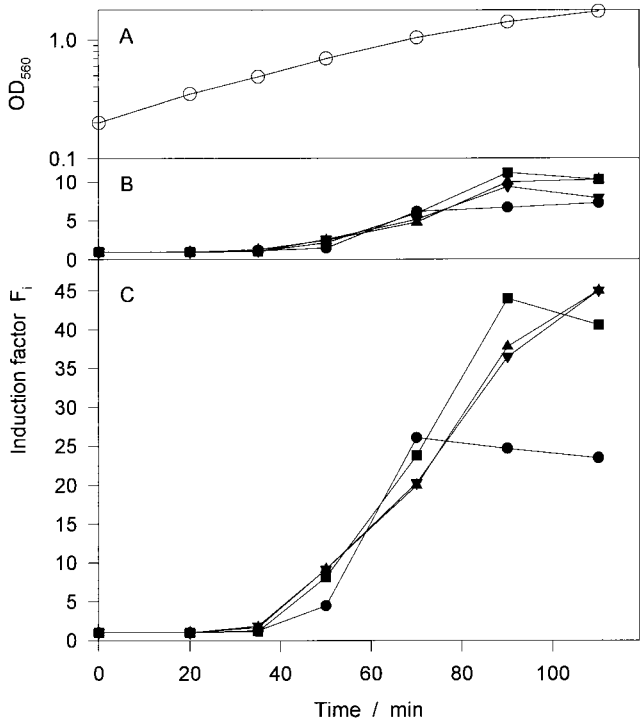

FIG. 2. Kinetics of SOS *lux* induction by MMC. Exponentially growing cultures of *E. coli* C600(pPLS-1) in L medium at an OD_{560} of 0.2 were treated with 100 (B) or 500 (C) nM MMC. The induction factor, F_i, and the cell concentration (OD_{560}) were determined after different incubation intervals at 37°C, followed by 0 (●), 5 (■), 10 (▲), and 15 (▼) min of incubation at room temperature. For comparison, the growth of the untreated control is shown in panel A.

physiological phenomena including SOS repair and SOS-dependent bioluminescence, is induced. The kinetics of SOS-dependent bioluminescence are dependent on the bioavailability and dose of the genotoxic agent as well as on a variety of cultivation conditions, such as concentration of the inoculum, composition of the growth medium, oxygen supply, and growth temperature. Figure 2 gives an example of the kinetics of SOS *lux* induction after incubation of the bioassay mixture with MMC at 37°C followed by different periods of incubation at room temperature. We have chosen this sequence of two temperatures during the incubation in order to optimize the light emission signal: first 37°C, which is optimum for SOS induction in *E. coli* cells, followed by room temperature, which allows cooling to optimum temperatures for bioluminescence in *E. coli* C600(pPLS-1), which are near 24°C (data not shown). It is well known that depending on the *Photobacterium* donor strain, stable bioluminescence in recombinant *E. coli* cells is mostly expressed at very narrow temperature ranges below 30°C (5).

Within the first 90 min of incubation, the bioluminescence signal continuously increased from the baseline level and then leveled off. A subsequent treatment at room temperature for 5 to 10 min was sufficient for the light signal to reach an optimum (Fig. 2B and C). Nearly identical induction curves were obtained for 10 and 15 min of the second incubation at room temperature. Therefore, we chose incubation periods of 90 min at 37°C followed by 10 min at room temperature for all subsequent tests for chemical genotoxins. To secure high reproducibility of the assay, these procedures were standardized as described in Materials and Methods.

During the incubation period the cell growth rate was nearly constant. Figure 2A shows the growth curve of the untreated control, which was not significantly different from that of the MMC-treated samples at these low concentrations (100 and 500 nM) of MMC (see also Fig. 3A).

Induction of SOS *lux* by chemical genotoxins. Light emission was induced in *E. coli* C600(pPLS-1) by six chemicals of known mutagenic potential (22, 27). These chemicals have different mutagenic mechanisms: MMC predominantly induces DNA intrastrand cross-linking, DNA base damage and strand breaks are induced by H_2O_2, alkylation of DNA bases is induced by MNNG and DMS, and NA inhibits DNA gyrase. UV and γ radiation, which were also tested, preferentially induce DNA base damage and strand breaks. The dose-response curves illustrating the genotoxic activities of the 6 chemicals are given in Fig. 3B. The degree of cytotoxicity is indicated by the impairment of cell growth (decrease in OD_x/OD_0, as shown in Fig. 3A). All tested genotoxins induced SOS-dependent bioluminescence progressively as the doses of the chemical under investigation were increased. At high concentrations, most substances were cytotoxic, leading to a decline in light emission with increasing doses. The concentrations of the genotoxin at which the SOS response was induced with minimum or no cytotoxicity (no remarkable decrease in OD_x/OD_0) ranged over 2 or more orders of magnitude, from 10 nM to 1 μM for MMC, from 0.5 to 50 μM for MNNG, from 5 to 100 μM for NA, and from 5 to 500 μM for DMS. At concentrations of the genotoxin lower than those used in this study, the bioluminescence signal was not distinguishable from that of the untreated controls and F_i was 1 (data not shown). MMC, NA, and DMS can be tested at even higher concentrations than shown in Fig. 3 without serious cytotoxicity. Exceptions were H_2O_2 and CH_2O, with small dynamic ranges for SOS induction (from 0.15 to 3 mM for H_2O_2 and from 0.3 to 0.75 mM for CH_2O) that were followed by significant cytotoxic effects at higher concentrations. For CH_2O, the highest value of F_i was 2 ± 0.2, at a concentration of 0.75 mM. At concentrations higher than 0.75 mM, CH_2O was highly cytotoxic and no light emission was observed ($F_i = 0$). It is interesting to note that MMC, MMNG, NA, DMS, and CH_2O give positive responses both in the SOS chromotest and the Ames mutatest (20, 30, 37), whereas H_2O_2 gives a positive response in the SOS chromotest but a negative response in the Ames mutatest (30).

The lower limit of detection, which is defined as that dose at which F_i reaches a value of 2, was derived from a linear plot of F_i versus dose. It was found that all tested chemicals met the requirements for genotoxicity as defined above. F_i increased by a factor of 2 or more, although CH_2O was just at the threshold of genotoxicity. For MMC the lower limit of detection was in the nanomolar range; for MNNG, NA, and DMS it was in the micromolar range; and for H_2O_2 and CH_2O it was in the millimolar range (Table 1). The limits of detection were thus well in agreement with those reported for other SOS assays, such as the SOS chromotest and *umu* test (Table 2). For the Ames mutatest, the lower limit of detection of the chemicals MMC, MNNG, and DMS was estimated from the reported revertants per nanomolar unit (27), taking into consideration the average values of background revertants. For these three chemicals, the Ames mutatest is more sensitive than the SOS assays.

Genotoxic potential was evaluated from the slope of the induction curves at low doses in a linear plot of F_i versus dose. MMC exerted the highest genotoxic potential, followed by MNNG, NA, DMS, H_2O_2, and CH_2O, in order of decreasing genotoxicity (Table 1).

The cytotoxic potential of the substrate was assessed from the relative optical density (OD_x/OD_0), which decreased with

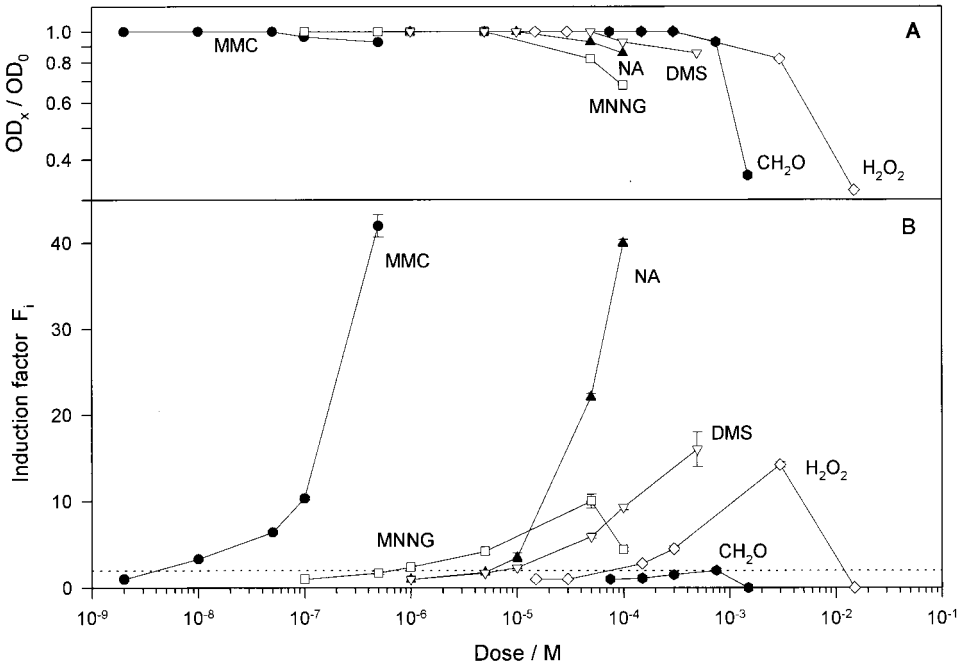

FIG. 3. SOS *lux* induction curves for MMC, MNNG, NA, DMS, H_2O_2, and CH_2O. The SOS *lux* induction factor, F_i (B), and the relative optical density, OD_x/OD_0 (A), were determined in cells of *E. coli* C600(pPLS-1) after incubation in the presence of the chemical for 90 min at 37°C followed by 10 min of incubation at room temperature. The dotted line is drawn at an F_i of 2. Where no error bars are shown, the size of the symbol is larger than the error bar.

increasing doses (Fig. 3A). To estimate the relationship between biomass and survival, in addition to the optical density the viable cell number, *N*, was determined by colony counting. Table 3 shows that after incubation with increasing doses of MMC, MNNG, and CH_2O, the survival rate (N/N_0) declined faster than the biomass (OD_x/OD_0). This is not surprising, because dead or nondividing cells also contribute to optical density.

As negative controls, AC, BUT, and CAM, which give negative responses in both the SOS chromotest and the Ames mutatest (20), were used; furthermore, TEC, which is nongenotoxic, although mutagenicity data are not available (20), and PHE were also used. Figure 4 shows the dose-response curves for these substances with respect to F_i and OD_x/OD_0. We applied dose ranges at which cytotoxicity started with increasing doses, as determined from the OD_x/OD_0 values (Fig. 4A). For PHE, TEC, and CAM, F_i decreased with increasing doses and did not significantly exceed a value of 1 (Fig. 4B). In response to AC and BUT, a slight increase of F_i with increasing doses, which dropped off at higher doses, was observed; however, all F_i values stayed well below 2. Therefore, none of the chemicals represented in Fig. 4 met the requirements for genotoxicity as defined above. The data demonstrate the specificity of the SOS *lux* test for genotoxins.

Induction of SOS *lux* by UV or γ radiation. UV irradiation (254 nm) showed high SOS-inducing potential in *E. coli* C600 (pPLS-1) (Fig. 5A), with a lower limit of detection at doses below 1 J/m^2 as determined from the linear plot of F_i versus dose (Table 1). The dynamic range of the SOS *lux* test spread from 0.3 J/m^2 to about 10 J/m^2. At doses higher than 10 J/m^2, OD_x/OD_0 was significantly reduced; however, even at a dose of 20 J/m^2, the SOS induction curve still increased. A comparable SOS response was observed after γ irradiation of *E. coli* C600 (pPLS-1) (Fig. 5B). The lower limit of detection was 2.6 Gy

TABLE 1. Sensitivity of the SOS *lux* test towards different genotoxins

Genotoxin	Genotoxic potential[a]	Lower limit of detection[b]
MMC	2.3×10^8/M	4.3×10^{-9} M
MNNG	1.4×10^6/M	7.1×10^{-7} M
NA	2.5×10^5/M	4.6×10^{-6} M
DMS	1.3×10^5/M	7.5×10^{-6} M
H_2O_2	1.2×10^4/M	8.3×10^{-5} M
CH_2O	1.6×10^2/M	6.5×10^{-3} M
UV (254 nm)	2.67 m^2/J	0.32 J/m^2
γ Rays	0.38/Gy	2.56 Gy

[a] Slope of the SOS response curves at low doses (from a linear plot of F_i versus dose).

[b] Dose of the genotoxin at which F_i reaches 2 (from a linear plot of F_i versus dose).

TABLE 2. Comparison of the sensitivity of the SOS *lux* test to some genotoxins with those of other mutagenicity or genotoxicity tests

Genotoxin (unit of measurement)	Lower limit of detection[a]			
	SOS *lux* test	SOS chromotest[b]	*umu* test[c]	Ames test[d]
MMC (M)	5.0×10^{-9}	1.7×10^{-8}	1.5×10^{-7}	2.3×10^{-10}
MNNG (M)	7.1×10^{-7}	5.0×10^{-7}	2.0×10^{-6}	3.6×10^{-9}
DMS (M)	7.5×10^{-6}	6.7×10^{-6}	3.0×10^{-4}	1.6×10^{-6}
γ Rays (Gy)	2.56	<5	ND	3–4

[a] Dose of the genotoxin or mutagen which increases the response by a factor of 2 over background levels.

[b] Data from references 27 (chemicals) and 29 (γ rays).

[c] Data from reference 22. ND, not determined.

[d] Data from references 27 (chemicals, estimated from the number of revertants per nanomolar unit) and 9 and 12 (γ rays).

TABLE 3. Cytotoxicity of selected chemicals after incubation of *E. coli* C600(pPLS-1)[a]

Substance	Concentration (M)	Cell density (OD_x/OD_0)	Survival (N/N_0)
Water		1.0	1.0
MMC	1×10^{-8}	0.98	0.99
	1×10^{-7}	0.93	0.70
	1×10^{-6}	0.88	0.31
MNNG	1×10^{-6}	0.98	0.99
	1×10^{-5}	0.94	0.81
	1×10^{-4}	0.64	0.01
CH_2O	3×10^{-4}	0.96	1.10
	7.5×10^{-4}	0.90	0.69
	1×10^{-3}	0.80	0.35

[a] Incubation for 90 min at 37°C plus 10 min at room temperature.

(determined from the linear plot of F_i versus dose), which is slightly lower than that of other mutagenicity tests (Table 2).

DISCUSSION

This study presents a genetically controlled luminescent bacterial reporter system for rapid screening of environmental genotoxins, the SOS *lux* test. The bioassay is based on the receptor-reporter principle, with the SOS system as the receptor sensitive to DNA damage and the bioluminescence system as the rapid optical reporter. We constructed the bioluminescent reporter plasmid pPLS-1, which carries the promoterless *luxCDABFE* genes of *P. leiognathi* downstream of a strong SOS promoter from ColD. For the SOS *lux* test, pPLS-1 can be used to transform any *E. coli recA*$^+$ strain or other microorganisms with an SOS-analogous system suitable for the detection of a certain genotoxic agent or mixtures thereof. A functioning *recA* gene is required to induce SOS function (31).

The SOS *lux* test is specific for the detection of genotoxins. Like other SOS-based bacterial tests, such as the SOS chromotest (27) and the *umu* test (23), it makes use of the fact that mutagenesis in *E. coli* cells exposed to radiation or chemical mutagens involves the SOS function, which is induced by DNA damage such as base changes or strand breaks (31, 38). Therefore, SOS-based systems also have been used for mutagenicity tests. By monitoring SOS gene expression, SOS bioassays specifically detect environmental genotoxic substances. The specificity of the SOS *lux* response was demonstrated for eight different mutagens. The spectrum of DNA damage induced by these mutagens ranges from DNA base modifications and alkylations to strand breaks and intrastrand cross-linking.

The SOS *lux* test was sensitive to the investigated genotoxins at concentrations as low as the nanomolar (MMC) or micromolar (MNNG, NA, and DMS) level. Its sensitivity was comparable to that of other bioassays, such as the other SOS bioassays mentioned and the Ames mutatest (Table 2). An example is given for γ rays: in the Ames mutatest, the lower limit of detection, i.e., the dose required to double the number of revertants over the spontaneous level, is in the range of 10 to 15 Gy for *S. typhimurium* TA98 and TA100 and 3 to 4 Gy for strain TA102 (9, 12). Similarly low detection limits are given for the SOS chromotest, with a range of 5 to 10 Gy (10, 11, 29). With 2.6 Gy for the SOS *lux* test, the detection limit was even lower than for the SOS chromotest. The sensitivity of the SOS chromotest increases for densely ionizing radiation like He ions (10). A similar trend was observed for the SOS *lux* test (unpublished data).

It has been shown by several authors that the results of SOS bioassays are largely comparable to those of the Ames test with regard to the genotoxic mutagenic potency and that 60 to 70% of genotoxic substances, as identified by SOS bioassays, also

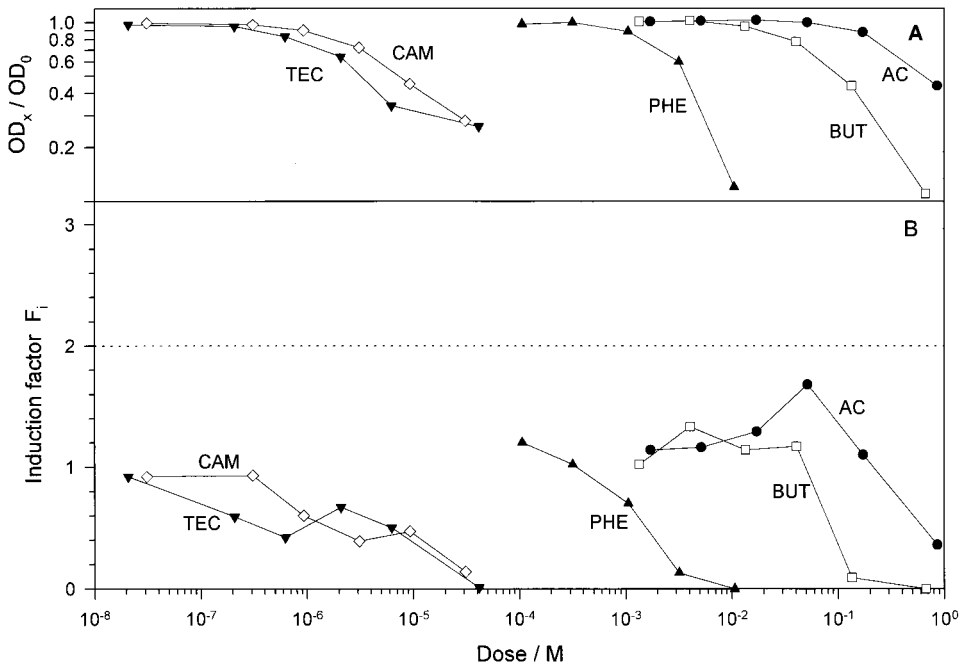

FIG. 4. Dose-response curves for AC, BUT, CAM, TEC, and PHE. The treatment was identical to that described in the legend to Fig. 3. The dotted line is drawn at an F_i of 2.

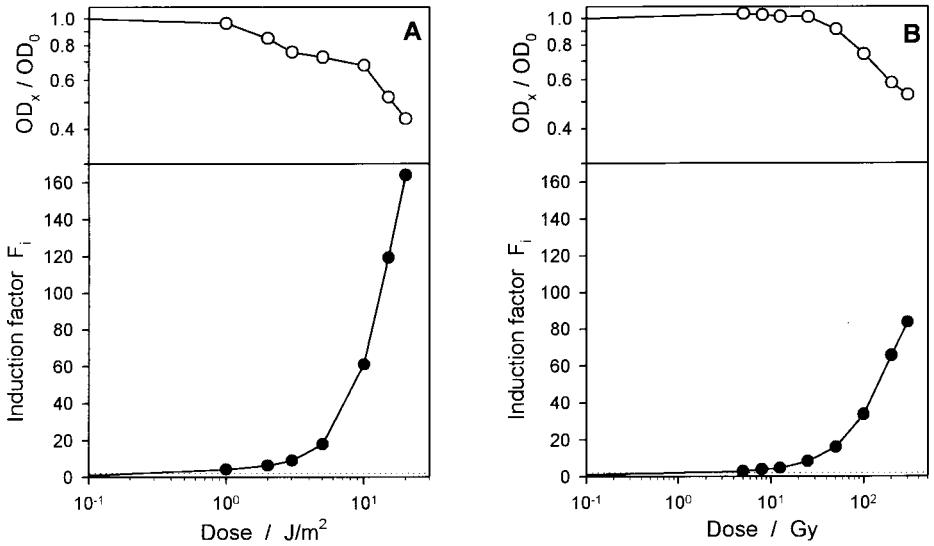

FIG. 5. SOS *lux* response to UV (254 nm) (A) and γ rays (B). The SOS *lux* induction factor, F_i, and the relative optical density, OD_x/OD_0, were determined in cells of *E. coli* C600(pPLS-1) after irradiation and incubation at 30°C for 120 min. The dotted line is drawn at an F_i of 2.

are carcinogenic for mammals (19, 22, 30). However, a recent quantitative evaluation showed a weaker correlation between genotoxicity determined by the SOS chromotest and carcinogenicity (37).

The SOS *lux* test, as compared with other bioassays for mutagenicity and/or genotoxicity, has a number of practical advantages. (i) The test results are available within 1 to 2 h. The registration time is very short (≤1 s). As a result, the SOS *lux* test is faster than any other bioassay for mutagenicity and/or genotoxicity. (ii) The bioluminescent reporter technology allows in vivo analysis without disruption of the cells. The light signal is taken from the living cell, and the measurement is nondestructive for the test bacteria and can be repeated several times. (iii) The SOS *lux* response can be measured repeatedly within a few seconds. This allows an increase in the precision of the data so that small deviations of F_i from a value of 1 can be detected (unpublished data). (iv) The bioluminescence data of the test culture can be monitored continuously during the incubation period. This allows the recording of the whole-time kinetics of SOS induction from the same culture. (v) Like other SOS bioassays, the SOS *lux* test detects a wide range of genotoxins with different DNA-damaging mechanisms, such as MMC, MNNG, NA, DMS, and H_2O_2, by using the same test strain. In contrast, different strains are required for the detection of the mutagenic potency of these agents with the Ames test. For example, MMC is detected by *S. typhimurium* TA102 but not by strain TA100 (owing to a defect in excision repair); H_2O_2 or ionizing radiation is effectively detected by strain TA102. (vi) Depending on the type of genotoxin, the sensitivity of the SOS *lux* test can be largely increased by using different host strains for the pPLS-1 plasmid. An example is UV radiation, for which *E. coli* strains deficient in excision repair are much more sensitive than the wild-type strain. Whereas the lower limit of detection was 0.3 J/m^2 with the wild-type *E. coli* C600(pPLS-1) strain, this value was lower by 1 order of magnitude with the *uvrA* mutant strain AB1886, which is deficient in excision repair (data not shown). Even higher sensitivity can be achieved by the use of the double mutant *umu uvr*. A similar increase in sensitivity is achieved for the SOS chromotest with an *E. coli* strain deficient in excision repair (28). Furthermore, the permeation of some chemicals can be improved by using a strain carrying a *rfa* mutation. Recently, a strain possessing high *O*-acetyltransferase activity was developed for the *umu* test, which is highly sensitive towards promutagenic aromatic amines (24). (vii) The simultaneous measurement of cell concentration and light emission allows a discrimination between the genotoxic and cytotoxic potency of the test substance to be made. If bioluminescence is not induced and the cell growth is comparable to that of the untreated control, the test substance is neither genotoxic nor cytotoxic. If, however, bioluminescence and/or OD_{560} decreases during incubation, then the test suggests the agent in question to be cytotoxic. The latter case was observed for CH_2O and H_2O_2 at higher concentrations, at which cytotoxicity exceeded the genotoxic effect (Fig. 3), and for CAM, TEC, PHE, BUT, and AC, which were used as negative controls (Fig. 4). In contrast, UV and γ rays were highly genotoxic even at high doses, which substantially inactivated the cells (Fig. 5).

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