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Microbial Sensors of Ultraviolet Radiation Based on *recA'::lux* Fusions

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Abstract

Escherichia coli strains containing plasmid-borne fusions of the *recA* promoter-operator region to the *Vibrio fischeri lux* genes were previously shown to increase their luminescence in the presence of DNA damage hazards, and thus to be useful for genotoxicant detection. The present study expands previous work by demonstrating and investigating the luminescent response of these strains to ultraviolet radiation. Several genetic variants of the basic *recA'::lux* design were examined, including a *tolC* modification of membrane efflux capacity, a chromosomal integration of the *recA'::lux* fusion, a different *lux* reporter (*Photorhabdus luminescens* instead of *V. fischeri*, allowing the assay to be run at 37°C), and a different host bacterium (*Salmonella typhimurium* instead of *E. coli*). Generally, two modifications provided the fastest responses: the use of the *S. typhimurium* host or the *P. luminescens lux* reporter. Highest sensitivity, however, was demonstrated in an *E. coli* strain in which a single copy of the *V. fischeri lux* fusion was integrated into the bacterial chromosome.

Index Entries: Bioluminescence; *Escherichia coli*; DNA damage; genotoxicity; microbial biosensors; *Photorhabdus luminescens*; *Salmonella typhimurium*; SOS response; UV irradiation; *Vibrio fischeri*.

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Introduction

The development of microbial bioassays for the detection of genotoxic hazards has drawn increasing attention in recent years. In addition to the well-established *Salmonella* reverse mutation assay (the Ames test) *(1,2)*, other approaches monitor activation of the bacterial SOS DNA repair system. Included in this category are the SOS Chromotest *(3)*, the Rec-lac test *(4)*, and the *umu* test *(5)*. In these cases, SOS induction of the *lacZ* reporter gene is followed using a chromogenic β-galactosidase assay at a single time point as a measure of genotoxicity. More recently, several reports have suggested detecting SOS activation by the use of bacterial bioluminescence (*lux*) as a reporter *(6–11)*, an approach that allows the monitoring of the bacterial response in real time by simple luminometry. Belkin et al.*(6,7)* and Vollmer et al. *(10)* pioneered the use, in *Escherichia coli*, of SOS-controlled promoters fused to *Vibrio fischeri*'s *luxCDABE* as genotoxicity indicators. In the Vitotox® assay *(9)*, the same plasmid-borne reporter system was fused to the SOS-regulated *recN* promoter of *E. coli* and introduced into *Salmonella typhimurium*. Ptitsyn et al. *(8)* have used, in *E. coli*, the fusion of the SOS-regulated *cda* promoter with the *luxCDABE* genes of *Photobacterium leiognathi*. Recently, Elasri and Miller *(12)* reported, in *Pseudomonas aeruginosa*, the fusion of its *recA* promoter to *V. fischeri luxCDABE* as a means for monitoring ultraviolet (UV) radiation, as previously demonstrated by Vollmer et al. *(10)* in *E. coli*.

In a recent study *(13)*, we compared the performance in the face of DNA-damaging chemicals of several modifications of the basic *(6,7,10) E. coli recA* promoter–*V. fischeri luxCDABE* plasmid-borne fusion strain. These modifications included incorporation of the fusion into the bacterial chromosome, the use of a different reporter system (*Photorhabdus luminescens luxCDABE*), the use of a *tolC* mutant *E. coli* host with impaired efflux capacity, or the substitution of *E. coli* with *S. typhimurium*. In the present article, we report on the response of these strains to UV radiation, probably the best studied and most extensively used model system for DNA damage *(14)*; UV-generated nucleotide dimers, the direct irradiation products, are known SOS-inducing agents *(15)*. Nalidixic acid, a chemical SOS inducer that inhibits DNA-gyrase and causes DNA double-strand breaks, was used as a positive control.

Materials and Methods

Bacterial Strains and Plasmids

Table 1 lists the different bacterial strains used. Their construction has been previously reported *(10,13)*.

Experimental Conditions, Measurement of Luminescence, and Data Analysis

Prior to the assay, the bacterial tester strains were grown overnight in LB broth *(16)* with shaking at either 26 or 37°C, and then diluted to approx

Table 1
Bioluminescent *recA'::lux* Bacterial Strains Used in This Study

Strain designation	Location of *lux* fusion[a]	*lux* origin[b]	*tolC*[c]	Host strain[d]	Assay temperature (°C)	Comments
DPD2794	mcp	Vf	+	Ec RFM443 *(21)*	26	
DPD2797	mcp	Vf	–	Ec DE112 *(19)*	26	
DPD1718	ci	Pl	+	Ec DPD1692	37	
DPD1714	ci	Pl	+	Ec DM800 *(22)*	37	
DPD1709	ci	Pl	+	Ec DM803 *(22)*	37	*lexA*^{ind}
DPD3063	ci	Vf	+	Ec W3110 *(23)*	26	
Sal94	mcp	Vf	+	St WG49 *(24)*	26	

[a]mcp, multicopy plasmid; ci, chromosomal integration.
[b]Vf, *V. fischeri*; Pl, *P. luminescens*.
[c]+, wild type; –, mutant.
[d]Ec, *E. coli*; St, *S. typhimurium*.

10^7 cells/mL and regrown under the same conditions until reaching a cell density of about 2×10^8. Kanamycin (25 mg/L) was included in the overnight growth medium, to ensure plasmid maintenance, but was omitted from the regrowth medium owing to an inhibitory effect on luminescence (S. Belkin, unpublished data). For the plasmid-bearing strains, repeated controls demonstrated no loss of kanamycin resistance, and hence no loss of plasmid, during the short assay period.

For the measurement of the effect of nalidixic acid, a twofold dilution series in LB broth was prepared in opaque white microtiter plates (Costar Europe, Badhoevedorp, The Netherlands), to a final volume of 50 μL in each of the wells. LB broth served as a control. To all wells, 50 μL of the early exponential cell suspension was added, and the plates were incubated in a temperature-controlled (26 or 37°C; *see* Table 1) microtiter plate luminometer (Lucy 1, Anthos Labtech, Salzburg, Austria).

For UV treatment, 100-μL cell aliquots in microtiter plates were subjected to irradiation from above at a constant intensity (2.6 J/[cm^2·min]) in a Vilber-Lourmat (France) Fluo-Link illuminator model TFL-35.M (6 × 15 W tubes, radiation peak at 312 nm). Different irradiation doses were obtained by varying the exposure period.

Bioluminescence values are presented as Lucy 1 arbitrary relative light units (RLU), or as the ratio of the luminescence of the induced sample to that of the uninduced control (response ratio) as described previously *(7,17)*.

All experiments were run in duplicate and were repeated at least twice (three times in most cases) at different dates, using different batches of cells. The standard deviation of duplicates was <5%.

Viability following UV or nalidixic acid treatment was determined by 24-h colony formation on LB agar plates.

Chemicals

All chemicals used were analytical grade. Nalidixic acid was obtained from Sigma (St. Louis, MO).

Results and Discussion

Kinetics of Light Production and Data Analysis

Light production in response to UV irradiation was dose dependent; under our experimental conditions, it peaked between 1 and 2 J/cm^2, whereas higher doses were inhibitory. In Fig. 1, the time course of luminescence development in strain DPD3063 (*see* Table 1) is presented in response to UV treatment (Fig. 1A) and to chemical challenge with nalidixic acid (Fig. 1B). The luminescent responses are also compared in Fig. 2, in which maximal luminescence levels are plotted as a function of inducer concentration. Also shown in Fig. 2 is the effect of both treatments on colony-forming ability of the cells. It can be observed that even at the lower doses, UV treatment was deleterious to cellular viability as judged by colony for-

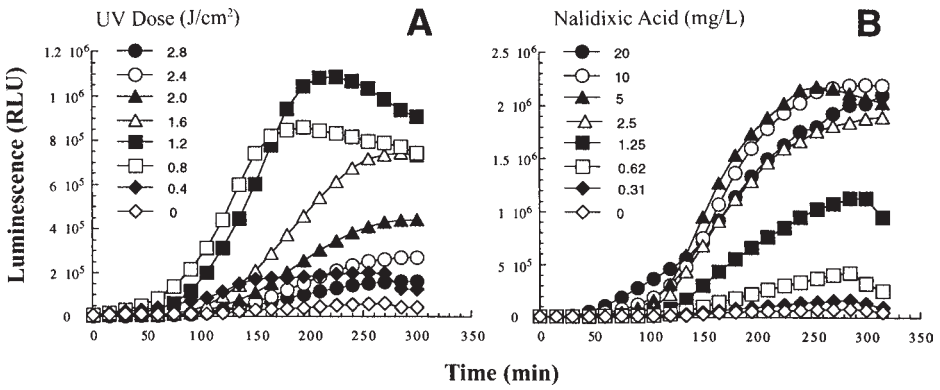

Fig. 1. Development of luminescence in the *recA'::lux*-harboring strain DPD3063 in response to **(A)** UV irradiation and **(B)** nalidixic acid.

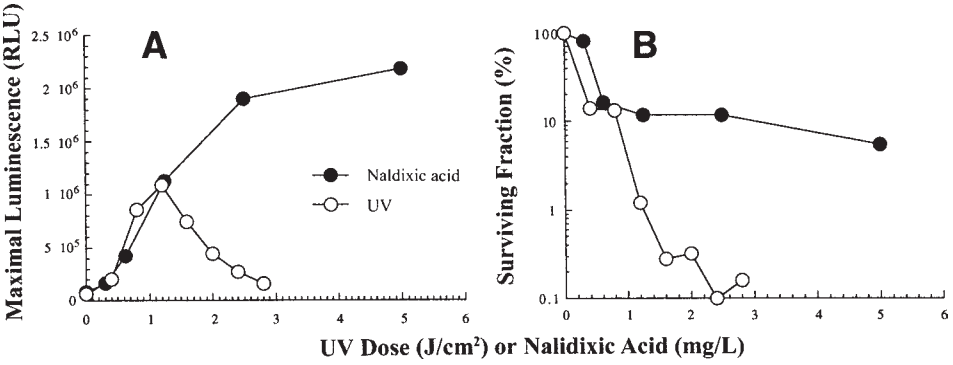

Fig. 2. Luminescent response **(A)** and survival of colony-forming ability **(B)** as a function of UV dose or nalidixic acid concentration. Data in (A) are peak luminescence values from Fig. 1.

mation on a solid medium. The ensuing luminescence following irradiation probably reflects the balance between enhancement owing to SOS induction of *recA* expression and inhibition owing to radiation damage. A similar phenomenon was observed also for nalidixic acid, although the survival of the population was larger and no significant inhibition of luminescence was observed in the concentration range tested. We do not have sufficient data at this time to determine whether the measured luminescence was generated by the "viable" colony-forming cells alone; it is clear, however, that above 1.2 J/cm^2 the negative effect of radiation had a stronger influence than did SOS induction.

Luminescent UV Response Is lexA *Dependent*

Figure 3 presents the response ratios (luminescence in the irradiated sample over that of the untreated control) as a function of UV dose in the two isogenic *E. coli* strains DPD1714 and DPD1709 (*[13]*; Table 1). Both

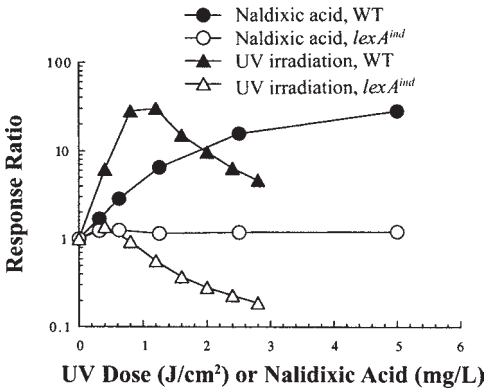

Fig. 3. Induction of *recA'::lux* requires LexA degradation: strain DPD1709 (*lexA*^{ind}, the mutant LexA repressor is refractile to degradation after DNA damage) bioluminescence is not induced by either UV irradiation or nalidixic acid. Strain DPD1714 is the isogenic *lexA* wild type (WT).

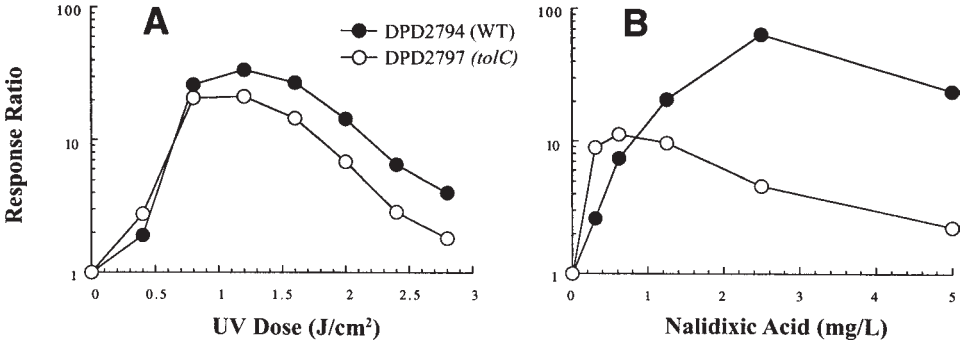

Fig. 4. A *tolC* mutation does not affect the response to UV treatment **(A)** but significantly increases sensitivity to nalidixic acid **(B)**. Strains DPD2797 and DPD2794 are isogenic except for the *tolC* mutation in the former.

harbor a chromosomal integration of *recA'::lux*, but the latter carries *lexA*^{ind} and thus contains a noncleavable form of LexA, the repressor of SOS gene expression *(14,15)*. As evident in Fig. 3, UV induction of luminescence was completely abolished when SOS activation was blocked by the refractile, noncleavable form of LexA.

A tolC *Mutation Does Not Affect UV Response*

It has been previously demonstrated that an *E. coli* host strain with a *tolC* mutation *(18)*, containing plasmids with *grpE'::lux (19)* or *recA'::lux (13)* fusions, significantly lowered the detection threshold of several chemicals. *tolC* mutants are impaired in their efflux capacity for certain compounds *(18)*, and the effect of the mutation should thus not be evident in the response to stimuli such as UV radiation. Indeed, as shown in Fig. 4A, the *tolC* mutant (strain DPD2797) exhibited the same UV sensitivity as its wild-

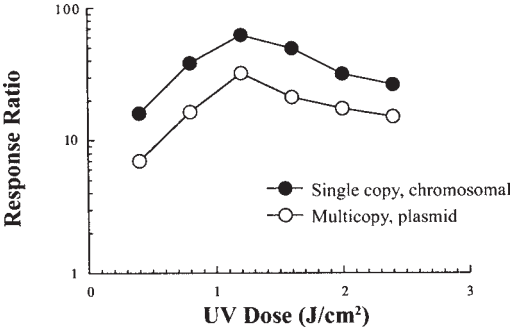

Fig. 5. Chromosomal integration of the *recA'::lux* fusion increases the response ratio. DPD2794, multicopy plasmid–based fusion; DPD3063, single copy chromosomal integration of fusion.

type isogenic partner (DPD2794). By comparison, the response to nalidixic acid of the two strains was strikingly different (Fig. 4B): that of the *tolC* mutant peaked at a 10-fold lower inducer concentration.

*Replicon for Promoter::*lux *Fusion: Multicopy Plasmid or a Chromosomal Integration*

The original bioluminescent genotoxicity sensor strains *(10)* harbored the *lux* fusion on a multicopy plasmid, thus enhancing the intensity of the observed response but introducing potential instability into the maintenance of the extrachromosomal genetic element. Another disadvantage of a multicopy plasmid-based fusion is possible loss of responsiveness to the regulatory element owing to a titration effect of hundreds of operator-promoters on a fixed level of repressor. Strain DPD3063 (Table 1, Fig. 1) chromosomally integrates a single copy of the same *recA'::lux* segment found in the plasmid-borne multicopy fusion strain DPD2794. Following UV irradiation, both strains exhibited similar luminescence kinetics, with the multicopy plasmid strain DPD2794 displaying higher luminescence intensities (not shown); the response ratios, however, were much more pronounced in the single copy chromosomal integrant DPD3063 (Fig. 5), mostly owing to the much lower basal level luminescence. An obvious effect of the increased response ratios is a lowering of the detection threshold.

Expanding Temperature Range by Use of P. luminescens lux *as Reporter*

One problem often accompanying the use of genetically engineered luminescent *E. coli* is the necessary compromise in working temperature: the optimal temperature of the *V. fischeri* luminescence enzymes is much lower than the 37°C required for "normal" *E. coli* functions. A solution may be provided by the *lux* system of *P. luminescens (20)*, which readily operates at this temperature. We have therefore compared the performance of two constructs: strain DPD1718, which contains a chromosomal integration of

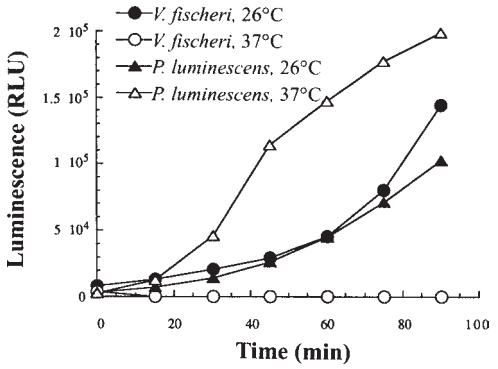

Fig. 6. Use of *P. luminescens lux* as a reporter allows a faster response. *V. fischeri lux*, DPD3063; *P. luminescens lux*, DPD1718. A UV irradiation dose of 1.2 J/cm^2 served as the inducer.

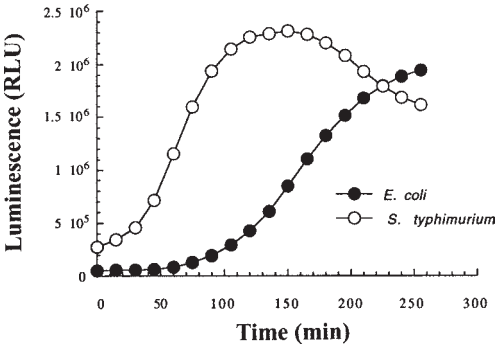

Fig. 7. Comparison of *S. typhimurium* (Sal94) and *E. coli* (DPD2794) as a host for the *recA'::lux* plasmid. A UV irradiation dose of 1.2 J/cm^2 served as the inducer.

luxCDABE genes from *P. luminescens*, and strain DPD3063, harboring the *V. fischeri* system. Figure 6 displays the first stages of the response to a single UV dose (1.2 J/cm^2) of the two strains at two temperatures. Performance at 26°C was similar, whereas at 37°C DPD3063 (*V. fischeri lux*) was nonfunctional. By contrast, DPD1718 had a clear advantage at this temperature, obviously conveyed by the different activity profile of the Lux proteins. This advantage, manifested also in response time, was somewhat offset by lower response ratios, which led to higher detection thresholds (not shown).

S. typhimurium, *an Alternative Microbial Host*

Although *E. coli* is a very convenient test organism, the use of *S. typhimurium* as a sensor strain seemed attractive owing to its general acceptance as a mutagenicity assay bacterium in the histidine reversion test *(1,2)*. *S. typhimurium* strain Sal94 was therefore constructed *(13)*, containing the same *recA'::lux*-bearing plasmid as *E. coli* DPD2794. Following UV treatment, Sal94 repro-

ducibly displayed a much faster initiation of luminescence. As an example, the reaction to a UV dose of 1.2 J/cm^2 is presented in Fig. 7. However, no significant differences in sensitivity were observed between the two constructs.

Conclusion

In view of the well-characterized and documented DNA damage hazards inherent in UV exposure, it was of interest to determine whether "bioavailable" radiation could be sensitively detected and assayed by bacterial sensor strains. Although physical devices can probably measure UV intensities at a much higher sensitivity and resolution than any bioassay developed to date, our approach provides a quite simple means by which the biological response to radiation can be monitored. The use of the basic tool—a *recA'::lux* fusion—for quantifying a cellular response to UV light has been presented before in *E. coli (10)* and in *P. aeruginosa (12)*. The present study assesses the effects of several modifications of the plasmid-borne system. The main conclusion to be drawn from the data is that a more rapid response may be obtained by the use of a higher assay temperature allowed by the *P. luminescens lux* system and by the use of *S. typhimurium*, rather then *E. coli*, as the host organism. Lowest detection limits were exhibited by a single copy chromosomal integrant of the *recA'::lux* fusion. Although further modifications in all system components may improve the performance of our microbial sensors, we believe that they already provide a unique tool by which UV radiation can be rapidly and sensitively biosensed.

Acknowledgments

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