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Responses to Toxicants of an *Escherichia coli* Strain Carrying a *uspA′:*lux Genetic Fusion and an *E. coli* Strain Carrying a *grpE′:*lux Fusion Are Similar

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Received 23 May 1995/Accepted 11 August 1995

A transcriptional fusion of the *Escherichia coli* *uspA* promoter to *luxCDABE* was characterized and compared with a heat shock-responsive *grpE*:lux fusion. Similarities in range and rank order of inducing conditions were observed; however, the magnitude of induction was typically greater for the *grpE*:lux fusion strain.

One approach to environmental monitoring is to detect changes in gene expression patterns induced by adverse conditions. Bacterial strains that increase light production in the presence of specific chemicals have been constructed by using the bacterial bioluminescence genes (*lux*) as reporters of transcriptional responses (2, 3, 12). A complementary approach, not requiring prior knowledge of expected contaminants, uses less-specific stress responses as general indicators of deleterious conditions. For example, a large variety of environmental challenges triggers the heat shock response (4). Accordingly, *Escherichia coli* strains containing the heat shock promoter *grpE*, *dnaK*, or *lon* fused to the *lux* reporter increase bioluminescence in response to many chemicals (13–15).

Monitoring induction of other stress-induced genes may also be useful for general detection of toxicants. Of particular interest is the *E. coli* *uspA* gene, which encodes the universal stress protein A (9). Conditions that limit cell growth, including nutrient starvation and exposure to toxic agents, induce *uspA* transcription (9, 10). A sequence characteristic of a σ^32^-activated promoter is found upstream of the apparent *uspA* mRNA start site in cultures exposed to carbon starvation (9) and heat shock (10). This promoter has little homology with the σ^32^-controlled heat shock response.

**Plasmids, *E. coli* strains, and bioluminescence quantitation.** *E. coli* TV1061 with plasmid pGrpELux5 has been described (14). The plasmid pUspALux2, containing a fusion of the *uspA* promoter region to the *Vibrio fischeri luxCDABE* genes, was constructed as previously described (14), using for PCR amplification the primers 5′-ACTTAAGGATCCCTCCCGATACGCTGCCA-3′ and 5′-AGCAGCGAATTCGGCGATGATCGCTGCCA-3′. Sequences from plasmids pGrpELux5 and pUspALux2 were identical to the promoter region sequences of *grpE* (5) and *uspA* (9), respectively.

The size and orientation of the inserted DNA were verified by PCR amplification of the inserted promoter region with the isolated plasmid DNA as the template and a primer, 5′-CTGTTCATTTAGGACAT-3′, from the *lux* region of pUCD615, paired with the previously specified upstream primer for *uspA* or the primer 5′-GAAGATTGATGACAA-3′ for *grpE*. The observed sizes of the amplification products corresponded with those predicted for the *grpE* and *uspA* fusions, 1,056 and 1,181 bp, respectively. This technique verified the initial constructs and confirmed the identity of the *uspA*:lux and the *grpE*:lux fusion strains from cultures used in independent induction experiments.

Light production was measured over an 80-min interval at 26°C with cells grown in LB medium (7) as previously described (14). Specific induction units (SIU), representing the increase in bioluminescence due to the presence of stress, normalized to the cells in the assay, were calculated as described previously (15).

**Induction of the *uspA*:lux fusion.** The *E. coli* strain containing the plasmid-borne operon fusion linking the *uspA* promoter to the *luxCDABE* reporter was characterized initially by challenge with several classes of chemical stressing agents. Figure 1 shows the kinetics of bioluminescence changes induced by various concentrations of *n*-propanol, phenol, copper sulfate, and sodium propionate. The presence of each of these compounds resulted in increased light output, with the increase being dependent on inducer concentration. This concentration dependence was bimodal in some cases (Fig. 1A, *n*-propanol, and Fig. 1B, phenol); concentrations below and above the optimal inducing concentration resulted in lower levels of light production. The decrease in bioluminescence at a high concentration of a stressing agent has been observed with other *lux* fusions (1, 3, 12, 14, 15). This decrease is likely due to the toxicity associated with inactivating Lux proteins or reducing metabolite levels, because light production requires...
active cell metabolism. Thus, the concentrations that yielded maximal induction were sublethal.

Comparison of responses of strains carrying uspA′::lux and grpE′::lux fusions. The induction of the uspA′::lux fusion by a variety of stresses was reminiscent of the heat shock-responsive grpE′::lux fusion (14). A direct comparison of E. coli strains containing these two fusions was made by using 25 stress conditions; the results are summarized in Table 1. The inducing agents are listed in order of maximum SIU of the uspA′::lux fusion strain. The rank order of the inducer strength was very similar to that observed with the grpE′::lux fusion strain. For both, alcohols tended to be the strongest inducers. Another similarity between the two strains was the range of compounds that resulted in bioluminescence induction. Extremely weak inducers (those with SIU of <0.1) and noninducers of both strains included mitomycin C, Triton X-100, sodium dodecyl sulfate, puromycin, and mercury(II) chloride. One exception to the similarity of responses was that UV irradiation, which was a very poor inducer of the uspA′::lux fusion strain, resulted in measurable induction of the grpE′::lux fusion strain. The degree of induction (SIU) was, in most cases, greater for strains containing the grpE′::lux fusion than for those containing the uspA′::lux fusion. When the lowest tested concentration that yielded a twofold increase in bioluminescence over the untreated control was used as an arbitrary estimation of the detection limit, the grpE′::lux fusion strain typically detected concentrations equal to or somewhat lower than those detected by the uspA′::lux fusion strain.

The many similar characteristics of stress induction between uspA′::lux and grpE′::lux fusion strains were unexpected, given the differing regulatory circuits controlling their expression. Alterations in plasmid copy number are unlikely to be responsible for the increases in bioluminescence because of the relatively short time of the stress induction experiment (one doubling time or less). Indeed, the plasmid content of cells containing a closely related plasmid having a different stress promoter is unaltered by three stresses (15). Several other explanations are possible for the similarities of the two fusions; further experimentation is required to distinguish among them.

The utility of the universal stress protein response of E. coli for detecting many environmental stresses was shown by using a fusion of the uspA promoter to the V. fischeri luxCDABE genes.
and test medium, may differentially effect the utility of the changes to maximize the sensitivity of these tests, such as the stress protein response should not be discounted because

For assessing induction by UV irradiation, cells in LB medium were irradiated at various doses with a Stratalinker UV Crosslinker

The extent of universally present in cells (8); hence, measurement of this response in bacteria may have relevance to all organisms. The extent of uspA conservation is not presently known, although DNA-DNA hybridization experiments indicate that some members of the family Enterobacteriaceae contain an analog of the uspA gene (16). Nevertheless, monitoring the universal stress protein response should not be discounted because changes to maximize the sensitivity of these tests, such as the use of permeability mutants (14) or alteration of the growth and test medium, may differentially effect the utility of the grpE::lux and uspA::lux fusions.

We thank Mary Bailey for synthesis of oligonucleotides.

REFERENCES


