Novel Cyanobacterial Biosensor for Detection of Herbicides

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The aim of this work was to generate a cyanobacterial biosensor that could be used to detect herbicides and other environmental pollutants. A representative freshwater cyanobacterium, *Synechocystis* sp. strain PCC6803, was chromosomally marked with the luciferase gene *luc* (from the firefly *Photinus pyralis*) to create a novel bioluminescent cyanobacterial strain. Successful expression of the *luc* gene during growth of *Synechocystis* sp. strain PCC6803 cultures was characterized by measuring optical density and bioluminescence. Bioluminescence was optimized with regard to uptake of the luciferase substrate, luciferin, and the physiology of the cyanobacterium. Bioassays demonstrated that a novel luminescent cyanobacterial biosensor has been developed which responded to a range of compounds including different herbicide types and other toxins. This biosensor is expected to provide new opportunities for the rapid screening of environmental samples or for the investigation of potential environmental damage.

Among the analytical methods used for environmental monitoring, microbial and cellular biosensors play an important role (11). Microbial biosensors have numerous advantages in ecotoxicity testing. Microorganisms are generally cheaper to culture than higher organisms, and they can be produced in large batches, subjected to stringent quality control procedures, and freeze dried for storage. They respond rapidly to toxic compounds and indicate the bioavailability of compounds in a way that chemical analysis cannot (21). Recent advances in genetic engineering have opened up new prospects in the field of microbial and cellular biosensors. An example is the use of the luciferase genes *lux* (from the marine bacterium *Vibrio fischeri*) (21) and *luc* (from the firefly *Photinus pyralis*) (12). These genes have been selected because there is a correlation between light output and the metabolic activity of the cell, so bioluminescence is a very rapid indicator of cell health (13, 21, 38). The genes encoding bioluminescence have been engineered into a range of microbes. Measurement of bioluminescence in response to exposure to samples can be used to assess the presence of a wide range of pollutants (21). Light itself is easy to measure in real time and lends itself well to automation, allowing the rapid processing of multiple samples. In addition, multiple or continuous readings can be taken, allowing both acute and chronic toxicity assessments to be made (23).

A variety of whole-cell-based bioluminescent biosensors have been constructed which respond to a wide range of pollutants while simultaneously assessing bioavailability in environmental samples (12, 16, 21, 22, 23, 41). However, existing biosensors show a poor response to herbicides, a key class of environmental pollutants (12). About one-half of the herbicides presently used in agriculture act by inhibiting the light reactions in photosynthesis, mostly by targeting the photosystem II (PSII) complex (10). A series of algal and cyanobacterial PSII-based whole-cell (5, 6, 11, 14, 27, 28, 34, 37, 43) and tissue (3, 15, 17, 18, 19, 30, 31, 32) biosensors have therefore been developed for detection of a class of herbicides which inhibit photosynthetic electron transport. In these systems, herbicides are detected by testing inhibition of the Hill reaction (6, 17, 31, 32), inhibition of 2,6-dichlorophenol indophenol photoreduction (4, 5, 15), or change in chlorophyll fluorescence (8, 11, 19, 30), which can be correlated with the pollutant concentration. Alternatively, the effect of herbicides was measured directly through inhibition of maximum growth rate (1, 34). However, the practical use of herbicide biosensors has so far been limited by their instability (15, 19), their short half-life (15, 19), the requirement for complex equipment (6, 15, 34), the time-consuming nature of the assays (15, 19), and the fact that most are specific for herbicides of the triazinephenylurea group, which inhibit photosynthetic electron transport (11, 15, 19).

Here we report the development of a cyanobacterial *Synechocystis* sp. strain PCC6803 biosensor, marked with the firefly *luc* gene, for detection of a broad range of herbicides and other pollutant classes including heavy metals and volatile organic pollutants by a simple and quick assay system. Cyanobacteria are unusual among prokaryotes in having two distinct membrane systems: the intracellular thylakoid membrane, which is the site of photosynthesis, and the more conventional eubacterial cytoplasmic membrane system around the cell. *Synechocystis* sp. strain PCC6803 was chosen as a potential biosensor for its extreme ecological importance and the availability of genetic tools. In this report we describe the sensitivity and rapid reaction of this novel cyanobacterial biosensor to herbicides in an acute assay format and compare its response to a well-characterized *Escherichia coli lux*-marked biosensor. Also, a novel chronic toxicity test system derived from this cyanobacterial biosensor is discussed.

MATERIALS AND METHODS

Strains and culture conditions. (i) *Synechocystis* **sp. strain PCC6803.** Wild-type *Synechocystis* sp. strain PCC6803 was obtained from laboratory stocks. It was grown routinely in BG-11 (29) liquid and solid medium at 30°C with constant

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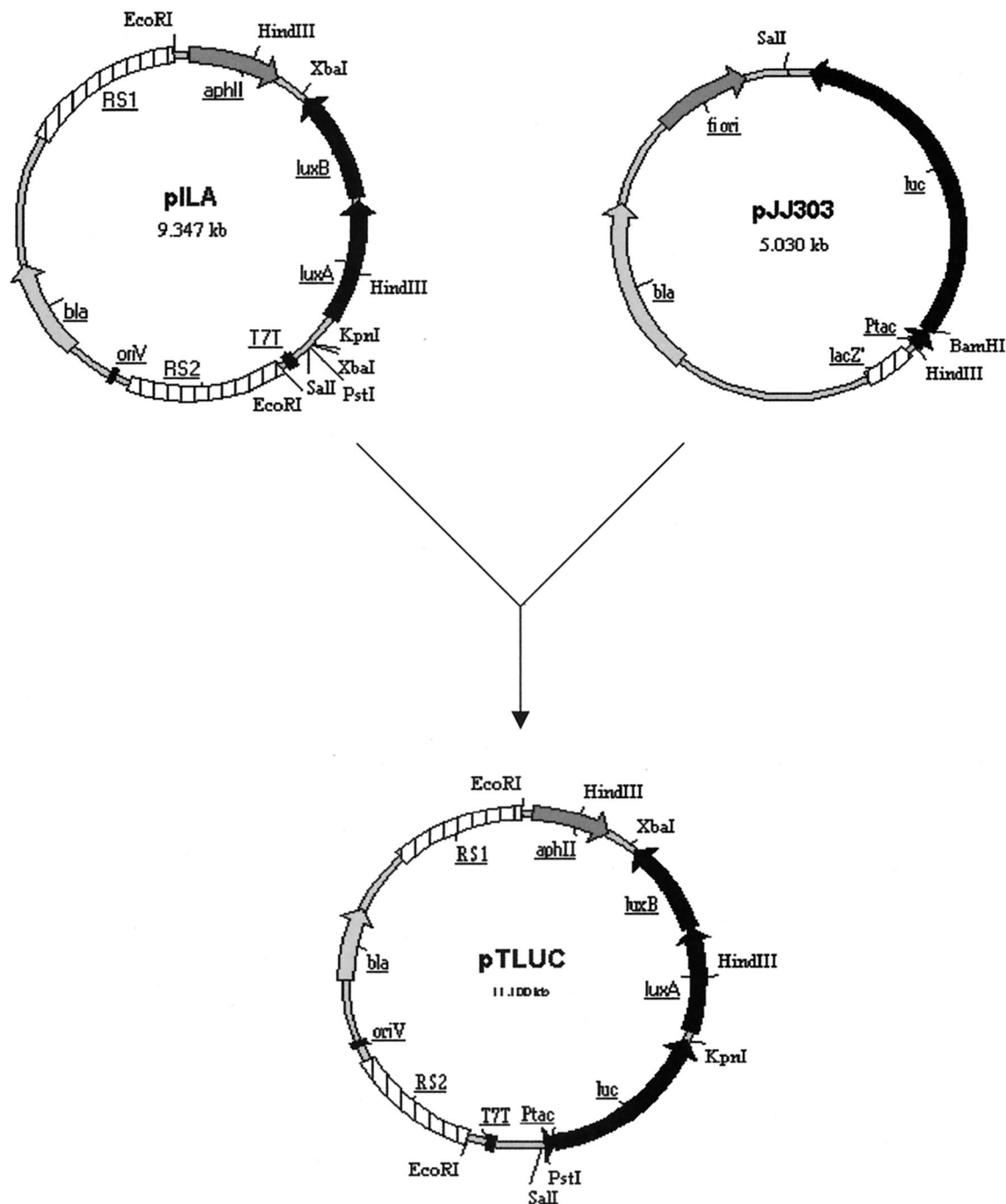
FIG. 1. Construction of plasmid pTLUC. The *Synechocystis* sp. strain PCC6803 integrative vector pILA (GenBank accession no. AJ251840) was used as a vector backbone for construction of pTLUC. A *tac-luc* PCR fragment, which introduced a *Pst*I site at the 5′ end and a *Kpn*I site at the 3′ end, was amplified by PCR from plasmid pJJ303 (20) and cloned into *Pst*I/*Kpn*I-digested pILA just upstream of the *luxAB* genes to create plasmid pTLUC. RS1 and RS2, a 3.0-kbp *Bam*HI fragment of *Synechocystis* sp. strain PCC6803 genomic DNA spanning the region from bp 31757 to bp 31793 (GenBank accession no. D63999).

illumination (25 μmol photons m^{-2} s^{-1} from cool-white fluorescent lamps). The antibiotic kanamycin (10 mg liter^{-1}) was included in liquid medium for culturing transformed cyanobacteria. The growth of cyanobacterial cultures was quantified by measuring the scattering of light (optical density) at 730 nm (OD$_{730}$) by use of a PU 8720 UV/VIS scanning spectrophotometer.

(ii) *E. coli*. Ultracompetent XL10-Gold cells supplied by Stratagene were used as the host for all cloning experiments. *E. coli* was grown in Luria-Bertani medium (33) and in the presence of kanamycin at 50 mg liter^{-1}.

Plasmids. Plasmid pILA (GenBank accession no. AJ251840), a derivative of the integration vector pKW1188 (42), was used as a vector backbone to create pTLUC (Fig. 1). A *Pst*I/*Kpn*I fragment containing the *luc* gene downstream of the *tac* promoter was amplified by PCR from the pGEM-*luc*-derived plasmid pJJ303 (20). Primer *tac*/*Pst*I (5′-TTACCTGCAGCTATTTAGGTGACACTATAG-3′) introduced a *Pst*I site at the 5′ end of the *tac* promoter region, and a *Kpn*I site was inserted by using primer *tac-luc*/*Kpn*I (5′-TTACGGTACCGACTTTCC GCCCTTCTTG-3′) at the 3′ end of the *luc* gene. This *Pst*I/*Kpn*I PCR fragment

TABLE 1. Herbicides used in this study

Herbicide	Type	Mode of action
Diuron	Phenylurea	PSII inhibitor
Atrazine	Triazine	PSII inhibitor
Propazine	Triazine	PSII inhibitor
Simazine	Triazine	PSII inhibitor
Paraquat	Bipyridyl	PSI inhibitor
Glyphosate	Phosphonomethyl glycine	Inhibits amino acid biosynthesis
MCPA	Phenoxy	Hormone mimic
Mecoprop	Phenoxy	Hormone mimic

was cloned into PstI/KpnI-digested pILA just upstream of the luxAB genes, resulting in plasmid pTLUC (Fig. 1).

Transformation of Synechocystis sp. strain PCC6803. To prepare a culture of Synechocystis sp. strain PCC6803 for transformation, cells were grown to an OD_{730} of approximately 0.6 (an OD_{730} of 0.25 corresponds to 10^8 cells ml^{-1}) and harvested by centrifugation at $4,500 \times g$ for 10 min at room temperature. The cell pellet was resuspended in fresh BG-11 medium at a density of 10^9 cells ml^{-1} and used immediately for transformation. Plasmid DNA (1 μg of pTLUC in 10 μl of 10 mM Tris-HCl [pH 8.5]) was added to 1 ml of cells, gently mixed, and incubated for 1 h with illumination at 30°C without agitation. Cells were spread on 50-ml BG-11 agar plates (0.1 ml per plate) and incubated under illumination for 2 days at 30°C. At this stage, the appropriate selective agent (kanamycin) was added by lifting the agar slab with an ethanol-flamed spatula and dispensing 50 μl of a solution containing kanamycin (10 mg ml^{-1}) underneath (7, 25). After 14 days, transformant colonies were isolated and purified by restreaking three times in the presence of antibiotics as above.

Preparation of herbicides and other pollutants. Stock solutions of the herbicides diuron, atrazine, propazine, simazine, paraquat, glyphosate, MCPA (4-chloro-2-methylphenoxyacetic acid), and mecoprop were prepared in sterile deionized water in acid-washed glass Duran bottles. Periods as long as 3 days with constant stirring were required to ensure complete solution of the herbicides, except for paraquat and glyphosate, which are very soluble. Fresh standard test solutions for these eight herbicides were prepared by diluting the stock solution with sterile deionized water in acid-washed glass Duran bottles. The test standards were used immediately after preparation.

The herbicides used in this study, with their modes of action, are listed in Table 1. The concentrations of herbicide stock solutions and the highest concentrations tested in this study are listed in Table 2.

Stock solutions of copper, zinc and 3,5-dichlorophenol (3,5-DCP) were prepared as previously described (21, 41). Fresh standard test solutions were prepared by diluting the stock solution with sterile deionized water.

Measurement of growth and luminescence characteristics. Correlation of luc-marked Synechocystis sp. strain PCC6803 cell growth and light output was studied by inoculating 1 ml of a late-exponential-phase culture ($OD_{730} > 6.0$) into 100 ml of BG-11 with 10 mg of kanamycin liter^{-1} in a 250-ml Erlenmeyer flask. Triplicate cultures were incubated on an orbital shaker at 150 rpm at 30°C with constant illumination, and 1-ml samples were taken at 24-h intervals for measurement of OD_{730} and luminescence. For luminescence analysis, 200 μl of culture was removed and 1 ml of McIlvaine's citrate phosphate buffer, pH 6.5 (10), containing 0.1 mM luciferin (Molecular Probes, Eugene, Oreg.) was added to the culture in each 1-ml luminometer cuvette. Following a 10-min exposure, bioluminescence was quantified in a BioOrbit 1251 luminometer by using a

Multiuse software package (version 2.01; BioOrbit, Turku, Finland). Luminescence was monitored over a 10-s period, and a mean was taken which was calculated by the software. Luminescence was expressed in relative light units (RLU), which equated to millivolts per 10 s per milliliter (26).

Effect of external pH on light output. To determine the effects of external pH on light output, a series of McIlvaine's citrate phosphate buffer (9) was prepared with a pH range from 2 to 8. Bioassays for determination of pH effect were based on the bioluminescence measurements described above, except that the pH of the citrate phosphate buffer was either 2, 3, 4, 5, 6, 7, or 8 rather than 6.5. Luminescence readings were taken at 10-min intervals for 130 min.

Bioassay procedures. A culture of luc-marked Synechocystis sp. strain PCC6803 was grown in 100 ml of BG-11 with 10 mg of kanamycin liter^{-1} in a 250-ml Erlenmeyer flask on an orbital shaker at 150 rpm at 30°C with constant illumination until peak luminescence was obtained (OD_{730} around 3.0). The cell suspension was then removed from the Erlenmeyer flask and centrifuged at 4,500 $\times g$ for 6 min. After two washes in sterile deionized water, the pellet was resuspended in 15 ml of sterile deionized water. A two-step bioassay procedure (12) was employed. A cell suspension (50 μl) was pipetted into each 1-ml cuvette (Clinicon; catalogue no. 2174 701) which contained 450 μl of the test solution. Following a variety of exposure times (for example, 10 or 30 min, 1, 2, 4, or 6 h, or 1, 2, 3, or 4 days for herbicides and 15 min for heavy metals and volatile organic pollutants), 500 μl of McIlvaine's citrate phosphate buffer, pH 6.5 (9), containing 0.1 mM luciferin was added at room temperature. Bioluminescence was then monitored over a 10-s period at room temperature in a BioOrbit 1252 luminometer by using a Multiuse software package (version 2.01; BioOrbit), and a mean was taken which was calculated by the software and expressed as RLU. Each assay was carried out in triplicate. The units of luminescence were then amended to RLU as before to allow comparison with the bioluminescence levels of lux-marked bacterial biosensor assays reported previously (39).

Statistical treatment of data. Bioluminescence, expressed as a percentage of that for a no-treatment control, was measured with each pollutant and plotted against the pollutant concentration for each of the time points, allowing effective concentrations (EC) to be established for each pollutant. The EC_{20} was taken as the effective concentration of pollutant needed to reduce bioluminescence by 20% from that of the control, while the EC_{50} corresponded to a 50% reduction in the bioluminescence signal. The EC_{20} and EC_{50} were calculated according to the work of Paton et al. (21).

RESULTS

Transformation of Synechocystis sp. strain PCC6803. Chromosomal transformation of Synechocystis sp. strain PCC6803 with the integration vector pTLUC (Fig. 1) was achieved by a natural transformation method (42). The vector backbone we used was an integrative vector (pILA; GenBank accession no. AJ251840) which contained a 3.0-kbp BamHI fragment of Synechocystis sp. strain PCC6803 genomic DNA (RS1 and RS2, representing the area from bp 31757 to bp 31793 in the Synechocystis sp. strain PCC6803 genome) (GenBank accession no. D63999). The tac-luc-luxAB-aphII genes were inserted into this fragment in pTLUC. After the transformation, the tac-luc-luxAB-aphII genes were integrated into Synechocystis sp. strain PCC6803 genomic DNA by a double-crossover event at the

TABLE 2. Toxic effects of eight herbicides on light outputs from luc-marked Synechocystis sp. strain PCC6803

Herbicide	Stock concn (mg/liter)	Highest concn used (mg/liter)	EC_{20}[a] (mg/liter) at:				EC_{50} (mg/liter) at:	
			30 min	6 h	1 day	2 days	30 min	1 day
Diuron	42	42	7.47 ± 0.61	NC	NC	NC	NDT	29.70 ± 0.62
Paraquat	3,750	370	0.95 ± 0.01	NC	NC	NC	2.85 ± 0.03	NC
MCPA	825	412.5	23.60 ± 0.02	NC	NC	NC	54.67 ± 0.16	NC
Mecoprop	620	310	12.57 ± 0.95	NC	NC	NC	47.55 ± 1.42	NC
Glyphosate	2,500	20	NDT	3.62 ± 0.79	NC	NC	NDT	3.10 ± 0.17
Atrazine	30	30	NDT	7.39 ± 0.95	NC	NC	NDT	NDT
Propazine	4.5	4.5	3.51 ± 0.05	NC	NC	NC	NDT	NDT
Simazine	4.5	4.5	NDT	NDT	NDT	1.06 ± 0.04	NDT	NDT

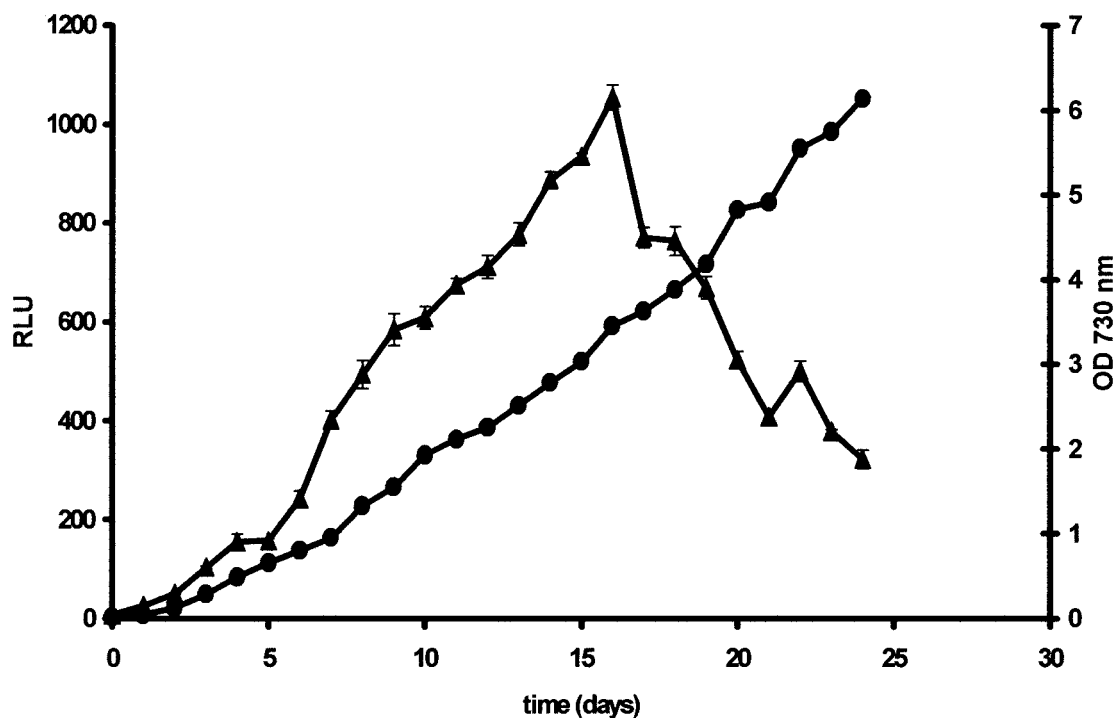[a] NDT, not detected; NC, not calculated.

FIG. 2. Light output during growth of *luc*-marked *Synechocystis* sp. strain PCC6803. Changes in OD_{730} (●) and luminescence (▲) of *Synechocystis* sp. strain PCC6803 during growth are means obtained from triplicate flasks ± standard errors of the means. For luminescence analysis, 200 μl of culture was removed and 1 ml of McIlvaine's citrate phosphate buffer, pH 6.5 (9), containing 0.1 mM luciferin (Molecular Probes) was added to the culture in each 1-ml luminometer cuvette. Following a 10-min exposure, bioluminescence was quantified in a BioOrbit 1251 luminometer by using a Multiuse software package (version 2.01; BioOrbit). Luminescence was monitored over a 10-s period, and a mean was taken which was calculated by the software. Luminescence was expressed in RLU, which equated to millivolts per 10 s per milliliter (26).

RS1 and RS2 sites. This result was confirmed both by PCR and by ampicillin sensitivity. For the PCR confirmation, we designed one primer using *Synechocystis* sp. strain PCC6803 genomic DNA information (which is not represented on plasmid pTLUC), and the other primer was designed to amplify from the *luc* gene. The PCR results indicated the integration of pTLUC plasmid DNA between the RSI and RS2 regions (data not shown). Similarly, the transformed strain was ampicillin sensitive and kanamycin resistant, indicating that the *bla* gene was not present (either through integration or as an episomal element) and that the *aphII* gene was present (through integration of pTLUC DNA between the RS1 and RS2 sites). The *luc* gene was inserted between bp 33429 and bp 33437 on the *Synechocystis* sp. strain PCC6803 chromosome (GenBank accession no. D63999). Sixteen different clones were analyzed for bioluminescence, and all gave very similar results, indicating a single, site-directed integration event.

Bioluminescence characteristics during batch growth. Light output during growth of transformed *Synechocystis* sp. strain PCC6803 in liquid batch culture is diagrammed in Fig. 2. Luminescence increased in parallel with biomass concentration until the OD_{730} reached 3 to 3.5 ($t = 16$ days) and decreased thereafter. However, even up to 24 days, when further testing was stopped, the OD_{730} continued to increase.

The growth rate of transformed *Synechocystis* sp. strain PCC6803 was not significantly different from that of the nontransformed parental strain (data not shown), indicating that integration and expression of the *luc* gene did not affect the growth rate. Similar results have been reported for *Pseudomonas fluorescens* (2, 35).

Effect of external pH on bioluminescence. Investigations performed to examine the effect of external pH on light output demonstrated that the external pH of the assay influenced light output drastically. At pHs between 2.0 and 5.0, bioluminescence peaked at 10 min and then decreased. At pH 8.0, bioluminescence showed the reverse trend; that is, it continued to increase with the length of the assay (Fig. 3). At the optimum pH of 6.5, bioluminescence showed a marked increase and light output remained stable over 2 h.

Toxicity of herbicides for *luc*-marked *Synechocystis* sp. strain PCC6803. Chromosomally *luc*-marked *Synechocystis* sp. strain PCC6803 was sensitive to a range of concentrations of all eight herbicides tested. The response was dependent on both toxin concentration and the length of exposure. As an example, Fig. 4 shows the effects of different concentrations of diuron at different lengths of exposure. The results show that the decrease in bioluminescence could be correlated with the herbicide concentration and with increasing incubation time. The toxicity values (EC_{20} and EC_{50}) detected with the shortest exposure times are listed in Table 2.

The biosensor was sensitive to the urea-based herbicide diuron (Table 2). The EC_{20} (7.47 ± 0.61 mg liter^{-1}) was obtained at 30 min, while 1 day was needed to obtain an EC_{50} (29.70 ± 0.62 mg liter^{-1}). The EC_{20} and EC_{50} decreased as exposure time increased; at 4 days, the EC_{20} was 4.08 ± 0.07 mg liter^{-1} and the EC_{50} was 10.85 ± 0.16 mg liter^{-1} (data not shown).
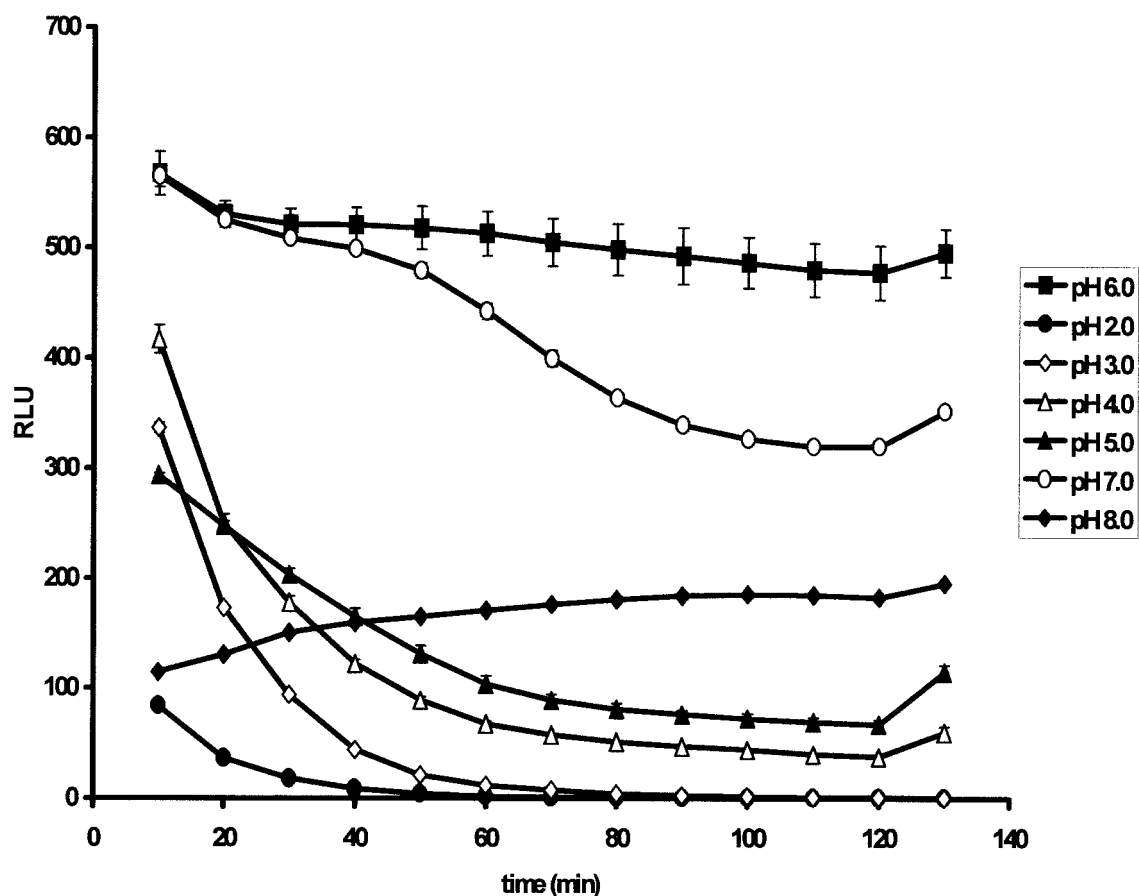
FIG. 3. Effects of external pH on bioluminescence responses after 10- to 130-min exposures. At pHs between 2.0 and 5.0, bioluminescence peaked at 10 min and then decreased. At pH 8.0, bioluminescence showed the reverse trend; that is, it continued to increase with the length of the assay. At the optimum pH of 6.0 to 7.0, bioluminescence showed a marked increase and light output remained stable over 2 h.

For the triazine-derived herbicides atrazine, propazine, and simazine (Table 2), EC_{20}s (7.39 ± 0.95, 3.51 ± 0.05, and 1.06 ± 0.04 mg liter^{-1}, respectively) were obtained at 6 h, 30 min, and 2 days, respectively. The toxicity of triazine type herbicides increased slightly with increasing exposure time, but even with an exposure time of 4 days, an EC_{50} could not be determined for the range of concentrations tested (data not shown).

Paraquat (Table 2) was the most toxic of all the herbicides tested. Both the EC_{20} (0.95 ± 0.01 mg liter^{-1}) and the EC_{50} (2.85 ± 0.03 mg liter^{-1}) could be determined at 30 min. The toxicity of paraquat increased rapidly with increasing exposure time (data not shown).

The phenoxy derivative herbicides MCPA and mecoprop (Table 2) were the least toxic of all the herbicides tested. However, the EC_{20}s (23.60 ± 0.02 and 12.57 ± 0.95 mg liter^{-1}, respectively) and EC_{50}s (54.67 ± 0.16 and 47.55 ± 1.42 mg liter^{-1}, respectively) could be easily obtained at 30 min of exposure. Increasing toxicity was also observed for this group of herbicides as exposure time increased (data not shown).

The EC_{20} and EC_{50} of the herbicide glyphosate were determined at 6 h and 1 day (Table 2), respectively. The EC_{20} at 6 h was 3.62 ± 0.79 mg liter^{-1}, and the EC_{50} at 1 day was 3.10 ± 0.17 mg liter^{-1}. The toxicity of glyphosate increased greatly with the exposure time; at 1 day, the EC_{20} was reduced to 0.41

± 0.11 mg liter^{-1}. The EC_{50} was much lower at day 2 (0.98 ± 0.02 mg liter^{-1}) than at 1 day.

Toxicity of heavy metals and a volatile organic pollutant. The cyanobacterial biosensor developed in this study was also very sensitive to nonherbicide toxicants, such as the heavy metals copper and zinc and a representative volatile organic, 3,5-DCP. The EC_{50}s, 0.24 ± 0.05, 0.88 ± 0.02, and 23.39 ± 0.45 mg liter^{-1}, respectively, were obtained at 15 min (Table 3).

DISCUSSION

In this study, a novel *luc*-marked cyanobacterial biosensor was developed for detection of a range of classes of herbicides with different modes of action (19, 30). The biosensor described in this study has been dually marked with *luc* and *luxAB*, and both *luc* and *luxAB* could be expressed in *Synechocystis* sp. strain PCC6803. When we attempted to measure expression of the *luxAB* genes by adding the bacterial luciferase substrate aldehyde, which is required to measure luminescence, we found that aldehyde was toxic to *Synechocystis* sp. strain PCC6803, even at very low concentrations (data not shown). However, firefly luciferase was suitable as a reporter to detect the toxicity of pollutants. The light reaction catalyzed by
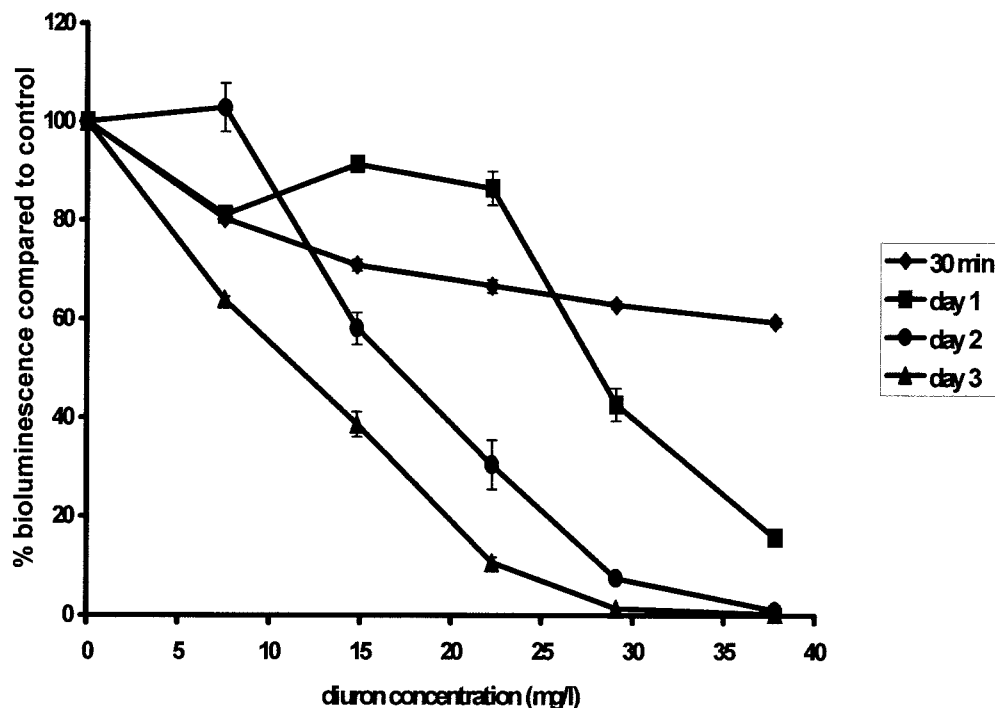
FIG. 4. Luminescence responses of *luc*-marked *Synechocystis* sp. strain PCC6803 cells after 30 min (◆), 1 day (■), 2 days (●), and 3 days (▲) of exposure to a range of diuron concentrations. The decrease in bioluminescence could be correlated with the herbicide concentration and with increasing incubation time.

the firefly luciferase enzyme relies on ATP being supplied by actively metabolizing cells. This dependence on endogenous energy supplies enables a luciferase assay system to report directly on cell health upon exposure to toxins. The optimum pH for assaying bioluminescence was found to be 6.5. This may be due to two factors: first, it may be the pH which supports the optimum physiology of the cyanobacterial biosensor, and second, it may be the pH at which the cyanobacterium is most permeable to the luciferin substrate.

When assay conditions (such as pH) were optimized, the cyanobacterial biosensor produced reproducible responses to a wide variety of herbicides (Table 2). Only one other study (39) has reported the effects of all the herbicides we tested except glyphosate on the bioluminescence response of *lux*-marked bacterial biosensors. The cyanobacterial biosensor was much more sensitive to the range of herbicides tested than the *lux*-marked *E. coli* HB101 biosensor (39). The cyanobacterial biosensor sensed the toxicity of herbicides at the EC_{50} level for four (all except the triazine class) of the five groups tested and at the EC_{20} level for all five of the herbicide groups tested. In contrast, the *E. coli* HB101 biosensor detected toxicity at the EC_{50} level for only two herbicide classes (paraquat and triazine) of four tested (glyphosate not tested) and at the EC_{20} level for three types (all except the urea type) of four tested (39). Furthermore, the cyanobacterial biosensor was more sensitive to the toxicity of herbicides than the *E. coli* HB101 biosensor; the EC_{50}s and EC_{20}s detected with the cyanobacterial biosensor were much lower than those with the *E. coli* HB101 biosensor.

The cyanobacterial biosensor reacts with the urea type herbicides diuron and paraquat more rapidly and more sensitively than an *E. coli* HB101 biosensor. The reason for this could be related to the modes of action of diuron and paraquat, which exercise their toxicity by targeting PSII (24) and photosystem I (PSI) (36), respectively. Thus, it is not surprising that diuron and paraquat were more toxic to the cyanobacterial biosensor, which has both photosystem, than to the *E. coli* biosensor, which has no photosystem. With respect to environmental relevance, the cyanobacterial biosensor is sensitive to herbicides at the parts-per-million level, which is appropriate for detecting residues in groundwater or soil, and in addition, the biosensor provides information on the bioavailability of the herbicide in environmental samples.

TABLE 3. EC_{50}s of Cu, Zn, and 3,5-DCP for a range of bioluminescence-based biosensors

Biosensor	EC_{50} (mg liter^{-1}) at 15 min		
	Cu	Zn	3,5-DCP
Synechocystis sp. strain PCC6803 (*luc* + *luxAB*)	0.24	0.88	23.39
Pseudomonas fluorescens 8866 (*luxCDABE*)[a]	0.30	0.10	4.82
Pseudomonas putida F1 (*luxCDABE*)[a]	0.17	0.04	5.55
Pseudomonas fluorescens 10586s (*luxCDABE*)[b]	0.09	0.09	NA[c]
Pseudomonas fluorescens 10586s (plasmid *luxABE*)[b]	0.76	0.89	NA
E. coli HB101 (*luxCDABE*)	1.4[d]	0.15[e]	18.5[d]

[a] Data from Weitz et al. (41).
[b] Data from Paton et al. (21).
[c] NA, data not available.
[d] M. P. Yeo (Aberdeen University), personal communication.
[e] A. M. Horsburgh (Aberdeen University), personal communication.

An assay period of 30 min was suitable for detection of most of the herbicides studied. The phenoxy acid herbicides MCPA and mecoprop interfere with systemic hormone signaling pathways. They affect cellular division, activating phosphate metabolism and modifying nucleic acid metabolism (40). From the results of this study, the cyanobacterial biosensor is more sensitive to MCPA and mecoprop than the *E. coli* biosensor (39) and a green algal biosensor (EC_{50}, 70 mg liter^{-1}; $t = 4$ days) (1). In comparison with other herbicides, the phenoxy acid herbicides were less toxic to the cyanobacterial biosensor, but an EC_{20} and EC_{50} could still be easily determined because of their high solubility.

Compared with the green alga *Selenastrum capricornutum*, the cyanobacterial biosensor is more sensitive to glyphosate in terms of reaction time and sensitivity. An EC_{50} of 1.05 mg liter^{-1} at 4 days was obtained with green algae (1), while an EC_{50} of 0.45 mg liter^{-1} was obtained at 1 day with the cyanobacterial biosensor developed in this study.

Herbicides of the triazine group, which includes atrazine, propazine and simazine, exert their toxicity by targeting PSII (24). The cyanobacterial biosensor could detect triazines at the EC_{20} level but could not be used to determine EC_{50}s for the triazines. One reason for this could be the low solubility of this herbicide group. If it had been possible to prepare test solutions of atrazine, propazine, or simazine at higher concentrations, it is highly likely that EC_{50}s would have been obtained (39). However, the biosensor does detect the bioavailability of these toxins, and solubility has a major impact on bioavailability. Instead of using EC_{50}s to describe the toxicity of the triazines, an EC_{10} might be a realistic indicator of the environmental health risk. Additionally, development of eukaryotic algal biosensors, which incorporate the *luc* gene driven by a specific inducible promoter, might provide a way to increase the sensitivity of the biosensor to chosen analytes.

The *luc*-marked cyanobacterial biosensor developed in this study was also very sensitive to nonherbicide toxicants, such as the heavy metals copper and zinc and a representative volatile organic, 3,5-DCP. Its sensitivity was competitive with those of a range of bioluminescence-based biosensors (Table 3).

In comparison with other bacterial biosensors, another advantage of the cyanobacterial biosensor is its suitability for monitoring the chronic toxicity of pollutants. The short doubling times of other bacterial and yeast biosensors make it difficult to detect chronic toxicity effects, because complications of cell death and potential cell division can confuse interpretation of results. The much longer doubling time of cyanobacteria makes it possible to predict the long-term effects of toxicants.

Conclusion. A novel luminescent cyanobacterial biosensor which responds to a range of compounds, including different herbicide types and nonherbicide toxicants, has been developed. The different herbicide types show different kinetics of bioluminescence inhibition. In comparison with other methods for detecting the toxicity of herbicides such as photosystem-based whole-cell and tissue biosensors (6, 11, 15, 19, 34), the whole-cell luminescent cyanobacterial biosensor proved to be more simple, rapid, accurate, and economical. The main application of this biosensor lies in rapid screening of samples or investigation of potential environmental damage. The biosensor could also be used to indicate the type of herbicide and possibly the potential mode of action.

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