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STATE-OF-THE-ART TECHNOLOGY USING GENETICALLY-ENGINEERED BIOLUMINESCENT BACTERIA AS ENVIRONMENTAL BIOSENSORS

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Bioluminescence is being used as a prevailing reporter of gene expression in microorganisms and mammalian cells. Bacterial bioluminescence draws special attention from environmental biotechnologists since it has many advantageous characteristics, such as no requirement of extra substractes, highly sensitive, and on-line measurability. Using bacterial bioluminescence as a reporter of toxicity has replaced the classical toxicity monitoring technology of using fish or daphnia with a cutting-edge technology. Fusion of bacterial stress promoters, which control the transcription of stress genes corresponding to heat-shock, DNA-, or oxidative-damaging stress, to the bacterial *lux* operon has resulted in the development of novel toxicity biosensors with a short measurement time, enhanced sensitivity, and ease and convenient usage. Therefore, these recombinant bioluminescent bacteria are expected to induce bacterial bioluminescence when the cells are exposed to stressful conditions, including toxic chemicals. We have used these recombinant bioluminescent bacteria in order to develop toxicity biosensors in a continuous, portable, or *in-situ* measurement from for air, water, and soil environments. All the data obtained from these toxicity biosensors for these environments were found to be repeatable and reproducible, and the minimum detection level of toxicity was found to be ppb (part per billion) levels for specific chemicals.

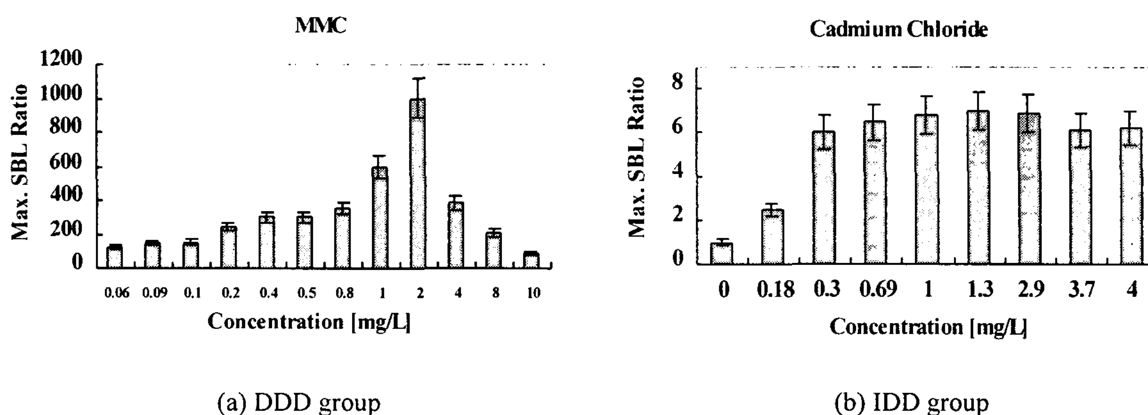
I. Toxicity chemicals measurable by using recombinant bioluminescent bacteria

The recombinant *Escherichia coli* strain DPD2794 containing a *recA::luxCDABE* fusion is used to detect genotoxicity of variou chemicals. Genotoxic agents were previously categorized into two groups, Direct DNA Damaging (DDD) agents and Indirect DNA Damaging (IDD) agents; these two groups have been distinguished with this strain. Minimum detectable concentrations of the DDD agents were about one to five orders of magnitude lower than those of the IDD agents. The response patterns of this strain to DDD agents differed from those to IDD agents in terms of kinetics and the forms of the dose-dependent response. The detection limit, the chemical concentration causing the response ratio (the maximum specific bioluminescence level (SBL)/control SBL) to be 2.5, for two groups of mutagents was measured using the strain DPD2794 (Table 1). Fig. 1 shows that the dose-dependent responses of the mutagens are also differentiated. For IDD mutagents, the response ratio remained highly and

fairly constant over a wide range of concentrations (Fig. 1b), resulting in plateau-type bar graphs. The dose-dependent responses of IDD mutagens were quite different from those of DDD mutagens in which a typical 'mountain-like' dose-response (Fig. 1a).

Table 1. Summary of the detection limit, the time of maximum SBL, and the response ratio at 100 min post induction. The response ratio of induced SBL_{max} to uninduced SBL_{max} tepresents the inducibility.

Chemical	Mutagens [Ref.]	Molecular Weight	Minimum Detectable Concentration, (ppb) Response ratio = 2.5	The time for SBL_{max} (concentration)	Response ratio at 100 minutes post induction	Response ratio of SBL_{max} at 230 minutes
DDD	Mitomycin C [5,6]	334	1.4×10^{-8}M (5 ppb)	230 min (2 ppm)	27.6 ± 2.20	1000 ± 80
	Methyl-nitro-nitrosoguanidine [8, 9]	180	3.3×10^{-8}M (6 ppb)	230 min (0.4 ppm)	77.4 ± 8.54	250 ± 30
	Benzo[a]pyrene [6]	252	5.2×10^{-11}M (0.013 ppb)	210 min (0.5 ppb)	9.07 ± 1.36	9.7 ± 0.15
	Gamma-ray [9]	-	1.5 Gy	300 min (200 Gy)	15.4 ± 1.38	20 ± 1.8
	Naphthalene	128	3.9×10^{-10}M (0.05 ppb)	210 min (1 ppb)	13.5 ± 1.62	8.9 ± 0.80
IDD	Hydrogen Peroxide [12, 15]	34.2	2.0×10^{-5}M (700 ppb)	210 min (10 ppm)	1.01 ± 0.15	8 ± 0.89
	Ethidium Bromide	394	3.2×10^{-7}M (125 ppb)	210 min (1 ppm)	1.78 ± 0.14	12 ± 1.8
	Cadmium Chloride [13]	183.32	9.9×10^{-7}M (183 ppb)	210 min (20 ppm)	1.87 ± 0.22	7 ± 0.77
	Methyl Viologen [25]	257.2	3.9×10^{-7}M (100 ppb)	210 min (1.3 ppm)	1.28 ± 0.14	11 ± 1.6
	Bisphenol A	228.28	2.2×10^{-6}M (500 ppb)	210 min (2 ppm)	1.08 ± 0.09	4.5 ± 0.49



(a) DDD group (b) IDD group

Figure 1. Dose-dependent response curves shown for mutagens of (a) the DDD group and (b) the IDD group.

II. A continuous on-line monitoring system using a two-stage minibioreactors for water toxicity monitoring

A two-stage minibioreactor system was successfully developed for continuous toxicity monitoring. This system consists of two minibioreactors in series (Fig. 2). The first stage allows for a continuous supplying of fresh cells to the second stage minibioreactor in which the biosensing cell and the sample water are mixed. To minimize the operation cost and space for the set-up of this system, small bioreactors were fabricated (working volume: 10 or 20ml). The minibioreactor has one side port with a glass for holding a fiber optic probe. The highly sensitive luminometer (Model 20e, Turner Design, CA) was linked to the other side of fiber optic probe to measure the bioluminescence in the minibioreactor. The luminometer was connected to a personal computer through a RS232 serial connection in order to acquire the real time data. Oxygen was supplied through a head port with a sparge tube by using pressurized air with flow meter at 1 v.v.m. Temperature was controlled by a water bath. After the steady-state values of constant bioluminescence and cell density obtained, the chemicals or test samples were injected into the second stage minibioreactor. If there are severe toxic chemicals injected into the second bioreactor, which will result in cell death, the system's ability to monitor toxicity will be recovered. The physical separation of cell culture allows stable and reliable operation of toxicity monitoring system, because cell growth rate and cell concentration can be constantly maintained in the first bioreactor with supplying into the second stage. This system can be also operated continuously with keeping of the responsiveness to toxic chemicals. The detection time and the detectable concentration are lower than other systems.

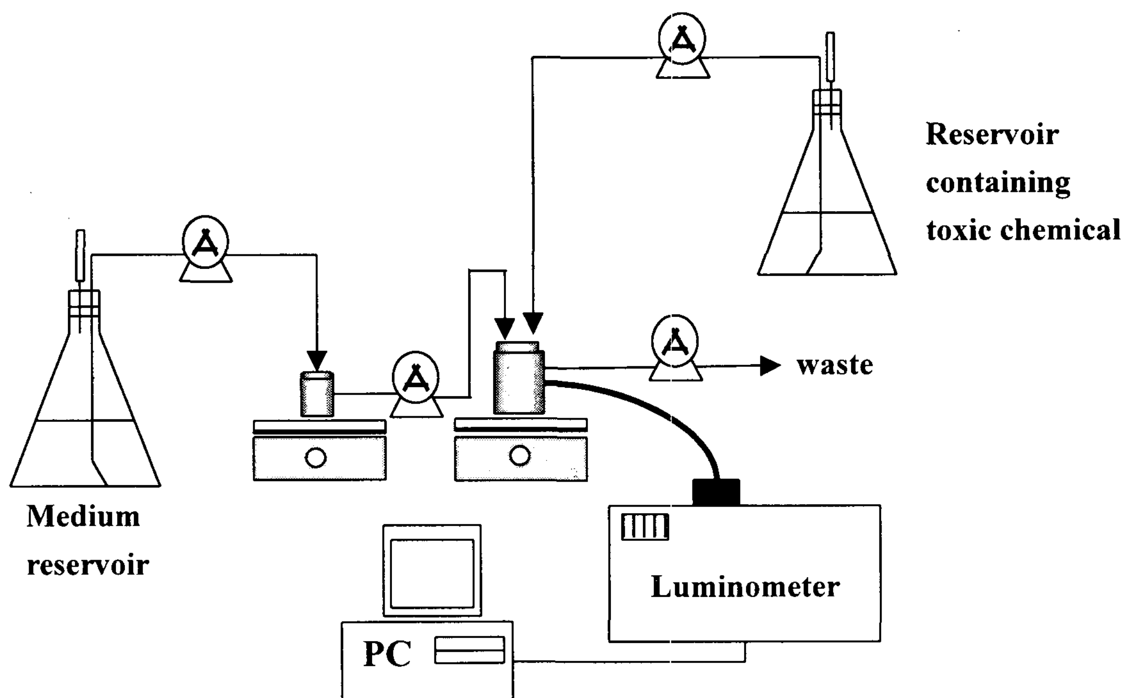


Figure 2. Two-stage continuous monitoring system

Recombinant *Escherichia coli* DPD2794 containing a *recA::luxCDABE* fusion as a model strain was utilized to monitor environmental insults to DNA, with mitomycin C as a model toxicant. And recombinant bacteria TV1061, containing a *grpE::luxCDABE* fusion, was used as a biosensor cell. This bacterial strain is responsive to toxicity due to protein-damaging agents. Pulse type exposures were used to evaluate the system's reproducibility and reliability. Step inputs of toxicants have been adopted to show the system's stability. All the data demonstrated that this two-stage minibioreactor system using recombinant bacteria containing stress promoters fused with lux genes is quite appropriate for continuous toxicity monitoring. Long-term operation and minimized media-usage have been investigated. Thus application to many different areas, including an early warning system of wastewater biotreatment plant upsets and the monitoring and tracking of accidental spills, discharges or failures in plant operation are plausible.

III. A gas toxicity biosensors using immobilized bioluminescent bacteria

A whole-cell biosensor was developed for the detection of gas toxicity using a recombinant bioluminescent *Escherichia coli* harboring a *lac::luxCDABE* fusion. Immobilization of the cells within LB agar has been done to maintain the activity of the microorganisms and to detect the toxicity of chemicals through the direct contact with gas. Benzene, known as a representative volatile organic compound, was chosen as a sample toxic gas to evaluate the performance of this biosensor based on the bioluminescent response. This biosensor showed a dose-dependent response, and was found to be reproducible (Fig. 3).

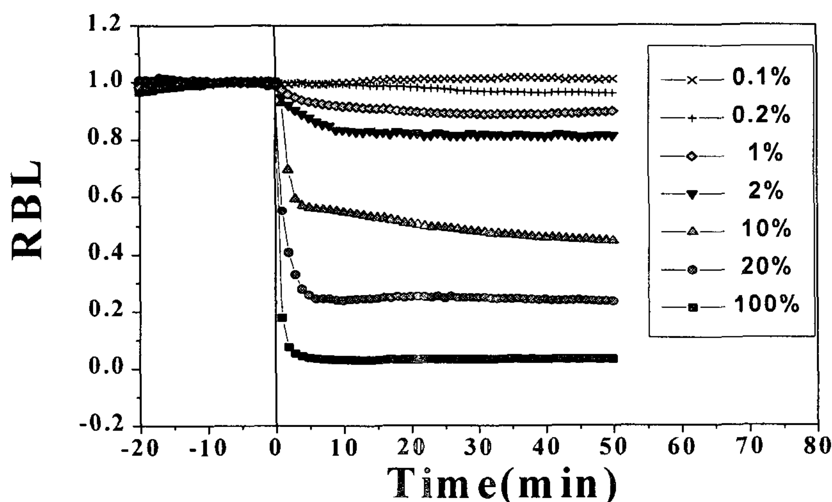


Figure 3. Response to different benzen concentrations from 0.1% to 100%.

The immobilizing matrices of this biosensor were stored at 4℃ and were maintained for at least a month without any noticible change in its activity. The optimal temperature for sensing was 37℃. A small size of this sensor kit has been successfully fabricated, and found to be applicable as a disposable and portable biosensor to monitor the atmospheric environment of a workplace in which high concentrations of toxic gases could be discharged.

IV. A soil biosensors using immobilized bioluminescent bacteria

A biosensor has been designed to detect a toxicity of polycyclic aromatic hydrocarbons(PAHs) in contaminated soil using immobilized recombinant bioluminescent bacteria, GC2(*lac::luxCDABE*). Polycyclic aromatic hydrocarbons(PAHs) are carcinogenic, mutagenic, and insoluble. In this study, phenanthrene, known as a representative PAH, was used as a sample toxic chemical. Biosurfactant was used to extract phenanthrene adsorbed on the soil surface. Biosurfactants can enhance the rate of mass transfer of PAHs from sorbed and the solid phase to the aqueous phase. And biosurfactants offer the advantage of being potentially less toxic. A recombinant bioluminescent bacterium, GC2(*lac::luxCDABE*), was immobilized with agar media and the glass beads to maintain the stability of its activity. This immobilized biosensor kit was connected with fiber optic probe in the mini-bioreactor maintained the constant temperature and aerated. Monitoring of phenanthrene was achieved by the measurement of decrease of bioluminescence when the extracted solution was injected into the mini-bioreactor. Immobilized biosensor kit could detect the toxicity of phenanthrene with different concentrations. The addition of glass beads in the immobilized biosensor kits enhanced the stability of the bioluminescence of the immobilized cells. This biosensor system may be applied as an *in-situ* biosensor to monitor toxicity of hydrophobic contaminants in soils and the efficiency of biodegradation systems of PAHs in soils

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