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A Whole Cell Bioluminescent Biosensor for the Detection of Membrane-Damaging Toxicity

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The recombinant bacteria strain DPD2540, containing a *fabA::luxCDABE* fusion, was used to detect the toxicity of various chemicals in this study. Membrane damaging agents such as phenol, ethanol, and cerulenin induced a rapid bioluminescent response from this strain. Other toxic agents, such as DNA-damaging or oxidative-damaging chemicals, showed a delayed bioluminescent response in which the maximum peak appeared over 150min after induction. This strain was also tested for measurement of toxicity in field samples such as wastewater and river water effluents.

Key words: *fabA* promoter, bioluminescent bacteria, *lux* gene, bioluminescent response, membrane damage

INTRODUCTION

Recently, the development of toxicity biosensors has drawn heavy attention from researchers since it was possible to create a gene fusion between the *lux* gene operon and stress promoters. These stress promoters regulate the synthesis of many different stress proteins on the transcriptional level, which help the cells adjust themselves to new environments when they are exposed to toxic or hazardous situations. A very wide range of different stress promoters are known to exist in the prokaryotic cell [1] and fusions of these stress promoters with the *lux* operon results in new cellular biosensing strains which emit light when induced.

Light emitting biosensors have several advantages [2]. One of the distinct merits of these biosensing cells is the readout, or bioluminescence, which can be measured *in vivo*. Bioluminescence has been defined as light emitted by a living organism arising by exergonic chemical reactions mediated by an enzyme [3]. The light emitting reaction in bacteria involves oxidation of a reduced flavin mononucleotide ($FMNH_2$) and a long-chain (7-16 carbons) fatty aldehyde in the presence of molecular oxygen [3]. The light emission from luminescent bacteria has been applied as a sensitive, rapid, and safe assay in several biological systems [3-5]. Some previous works also include characterization of these biosensing cells and fabrication of a miniature bioreactor for the continuous monitoring of toxicity of samples [6-8].

One of the recombinant bioluminescent strains, DPD2540, harboring the fusion gene operon *fabA::lux CDABE*, was recently constructed to detect toxicity caused by membrane-damaging pollutants [9,10]. *fabA* is a gene responsible for the formation of a double bond in fatty acids used in the membrane of *E. coli*, and is under the positive regulation of *fadR* [11,12]. FadR acts both as a repressor of genes regulating fatty acid degradation and as a transcriptional activator of fatty acid biosynthesis. In the event that fatty acid biosynthesis exceeds the amount needed for membrane production, long chain acyl-CoA molecules accumulate and bind to the FadR protein, dissociating it from the *fabA* promoter [11,12]. In the event of fatty acid starvation caused by membrane damage, long chained acyl-CoA pools will be low and expression of *fabA*, and the *fabA-lux* fusion as well, should be high [11,12]. Thus, DPD2540 may give a bioluminescent output as the cell membrane is damaged.

Therefore, in this study, DPD2540 was used to detect and characterize the toxicity caused by various chemicals including membrane-, DNA-, and oxidative-damaging agents. In addition, wastewater samples were also tested with this strain to demonstrate the possibility of the strain application in real situations.

MATERIALS AND METHODS

Strains, Culture Media, and Conditions

The recombinant bioluminescent strains *E. coli* DPD2540 harboring the plasmid pFabALux6/RFM443 was used in this study [9,10]. The plasmid pFabALux6 contains a fusion of the *E. coli fabA* stress promoter to the *Vibrio fisheri luxCDABE* genes and confers resistance to kanamycin and ampicillin. The medium used in this study was Luria-Bertani (LB), adjusted to pH 7 and supplemented with 25 mg/mL kanamycin monosulfate to maintain the plasmid. 100 mL seed cultures were grown in 250 mL flask with agitation at 300 rpm in a rotary incubator at 30℃. After sterilization, each flask was inoculated with 2 mL of inoculum from an overnight preculture in 100 mL of medium and grown under the same conditions as the seed cultures.

Measurement of Bioluminescence and Cell Growth

When the optical density at 600 nm (OD600) reached 0.08 (the early exponential phase), toxic chemicals were

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added to the culture. In real sample experiments, a 20% effluent wastewater sample was made by diluting 100% effluent wastewater from a chemical plant (BouWaters Hanla Pulp Co., Mokpo, Korea) and a 20% river water sample (near the city of Kwangju) was also prepared by the same method. In both wastewater and river water experiments, various phenol concentrations were applied. Bioluminescence of the culture, as a result of *fabA* induction, was measured at regular time intervals by a luminometer (Model 20e, Turner Design, CA, U.S.A.). Stress responses were measured by specific bioluminescence (SBL; Bioluminescence (BL)/Cell density (OD 600)). The optical density was measured at 600 nm using a spectrophotometer (Perkin-Elmer Co.)

Chemicals

Phenol, cerulenin and mitomycin C were purchased from the Sigma Company. Hydrogen peroxide and ethanol were purchased from the Merck Company.

RESULTS AND DISCUSSION

Bioluminescent Response to Membrane Damaging Chemicals

Ethanol is commonly used as an antimicrobial compound. Ethanol decreases membrane integrity by interacting with cell membranes. [13]. As shown in Fig. 1, a bioluminescent response was induced in DPD2540 by ethanol, with the degree of cytotoxicity indicated by the impairment of cell growth. Ethanol induced the *fabA*-dependent bioluminescent response progressively as the dose of the ethanol increased. At concentrations higher than 2%, ethanol was cytotoxic, leading to a decline in light emission and growth rate with increasing doses. Therefore, the cell growth and bioluminescent response should be measured concurrently, since the bioluminescent response did not represent the toxicity at higher concentrations of toxic chemical.

As shown in Fig. 1, when the cells entered the stationary phase, about 150 min after induction, the control showed a bioluminescent response with no chemical addition. This could be because the fatty acid composition of the cell membranes is changed as the cells enter the stationary phase [14], and acetic acid is also accumulated (data not shown). Thus, such membrane change induced by entering the stationary phase as well as by accumulated acetic acid could turn on the *fabA* promoter and result in the bioluminescent response without chemical induction.

Fig. 1(b) shows that DPD2540 displayed a two-peak bioluminescent response as a result of membrane damage by ethanol. The first peak appeared during the exponential phase and the second peak was detected in the stationary phase. To interpret the two-peak bioluminescent response, the change of the cell membrane dependant on the growth stage was considered. Ethanol was added at the early exponential phase (OD 0.08) and induced the *fabA* dependent bioluminescent response, that is, the first peak response during the exponential phase. As the cells entered the stationary phase, they gave a second bioluminescent response probably due to the cells entering the stationary

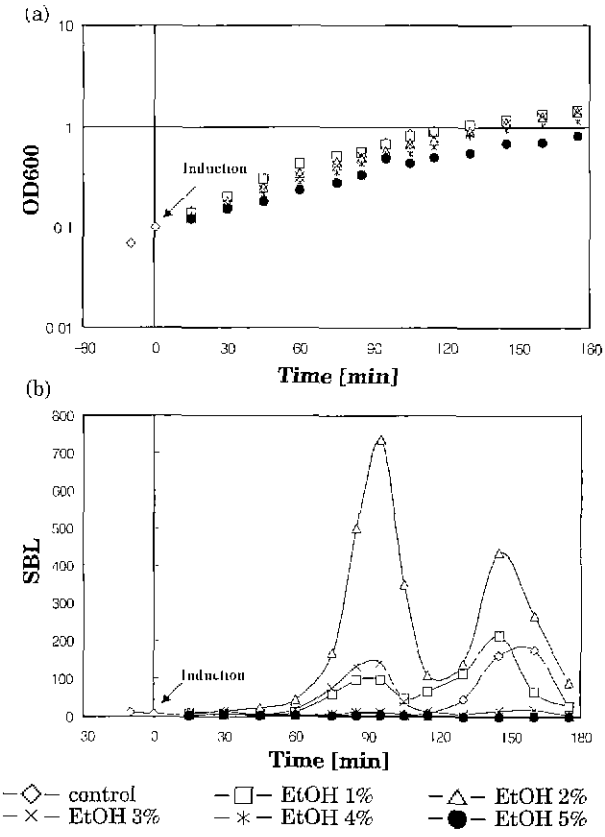


Fig. 1. Bioluminescent response and growth of DPD2540 with the addition of various ethanol concentrations; (a) time course of cell concentration, (b) time course of specific bioluminescence.

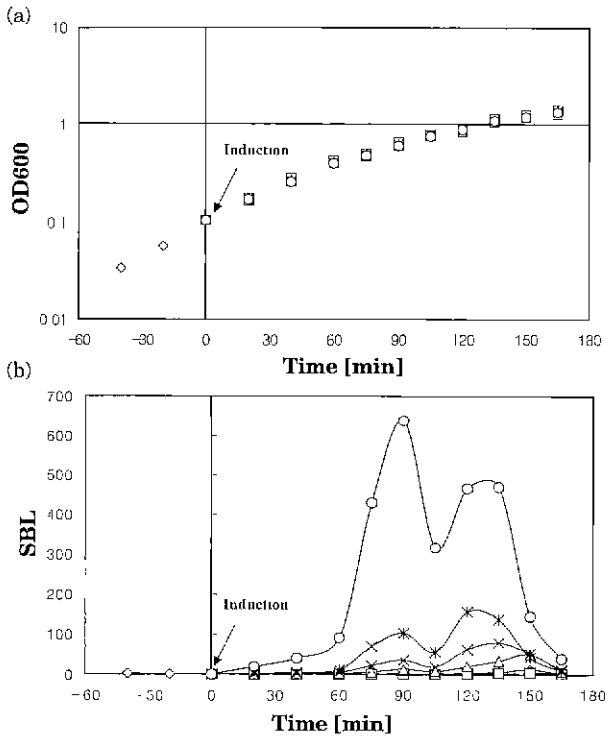
phase, residual damage from ethanol and accumulated acetic acid.

Other membrane damaging chemicals, such as phenol and cerulenin, showed a similar response pattern to that of ethanol, except for the concentration giving the maximum SBL (data not shown). The sublethal concentration (causing maximum SBL output) was 500 ppm for phenol and 75 ppm for cerulenin. It is known that phenol changes the membrane functioning and influences protein-to-lipid ratios in the membrane [13], while cerulenin inhibits the β-ketoacyl-ACP synthetase, which is a fatty acid biosynthesis enzyme [12].

Phenolic compounds are commonly found in wastewater originating from industry due to their relatively high aqueous solubility [13]. To show the possibility of using a biosensing bacterium in the real environmental ecosystem, real wastewater and river water samples were tested with this biosensing strain. As shown in Fig. 2, 20% of effluent wastewater (BouWaters Hanla Pulf Co., Mokpo, Korea) containing various phenol concentrations resulted in a bioluminescent response. DPD2540, however, was not responsive to the wastewater effluent with no phenol addition. River water, from the city of Kwangju, showed a similar response pattern (data not shown). These results show that the bioluminescent biosensing cell can detect an incident of phenol leakage from wastewater plants or chemical manufacturing plants.

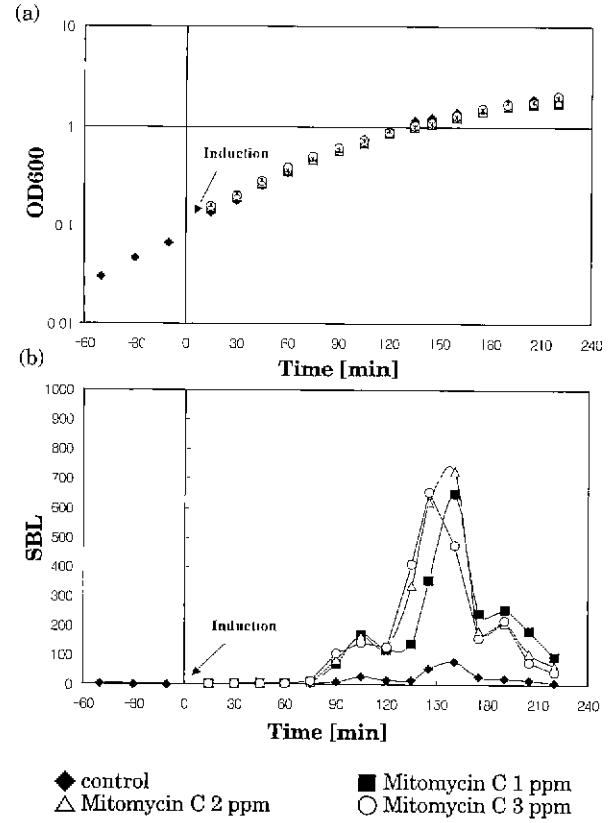
Bioluminescent Response to Other Damaging Agents

Mitomycin C (MMC) is a DNA damaging agent [15]



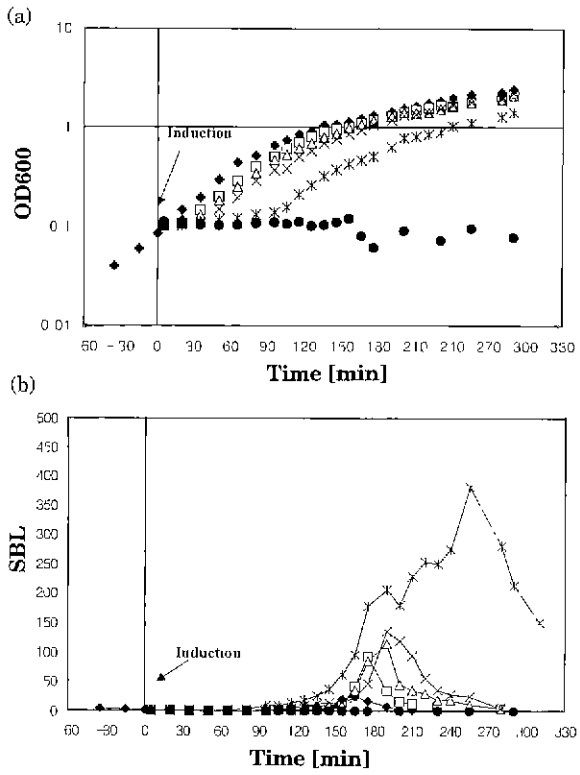
◇ control w/o wastewater □ control with wastewater
△ wastewater +phenol 57 ppm × wastewater +phenol 114 ppm
∗ wastewater +phenol 171 ppm ○ wastewater +phenol 342 ppm

Fig. 2. Bioluminescent response and growth of DPD2540 in 20% wastewater containing various concentrations of phenol; (a) time course of cell concentration, (b) time course of specific bioluminescence.



◆ control ■ Mitomycin C 1 ppm
△ Mitomycin C 2 ppm ○ Mitomycin C 3 ppm

Fig. 3. Bioluminescent response and growth of DPD2540 with the addition of various Mitomycin C concentrations; (a) time course of cell concentration, (b) time course of specific bioluminescence.



−◆− control −□− 0.0005% H_2O_2 −△− 0.001% H_2O_2
−×− 0.002% H_2O_2 −∗− 0.005% H_2O_2 −●− 0.01% H_2O_2

Fig. 4. Bioluminescent response and growth of DPD2540 with the addition of various H_2O_2 concentrations; (a) time course of cell concentration, (b) time course of specific bioluminescence.

that predominantly induces DNA intrastrand cross-linking, resulting in the cellular SOS response [15]. Contrary to the expectation that DNA damaging agents do not result in a *fabA*-dependent bioluminescent response, the experimental results showed that MMC produced a bioluminescent output, even though the response was delayed (Fig. 3).

H_2O_2, known to be an oxidative damaging chemical [16], caused a significant growth inhibition after addition but the growth was revived as time went on (Fig. 4). This is due to the fact that the inhibitive effect of H_2O_2 is reversible over time [17]. H_2O_2 also induced the *fabA*-dependent bioluminescent response (Fig. 4) as MMC did, again with a delayed response. This result was also not anticipated since H_2O_2 is not thought to cause membrane damage. For both MMC and H_2O_2, the exact mechanism for the induction of the *fabA* promoter is not known. However, in this case, the *lux* genes are thought to be expressed under the control of a global regulation response since both bioluminescent responses occurred in the stationary phase, during which the cell undergoes many intracellular changes.

These experimental results showed that, as a whole cell biosensor based on the *fabA-lux* gene fusion, DPD2540 could detect the *toxicity* of various hazardous chemicals including membrane-, DNA-, and oxidative-damaging agents. This strain may be able to separate toxic chemicals into two groups, direct-membrane damaging chemicals and indirect-membrane damaging chemicals. Based on the bioluminescent response pattern, a two-peak response for a direct-

damaging agent, such as ethanol, phenol, and cerulenin, and a single peak response during the stationary phase for an indirect-damaging agent, such as MMC and H_2O_2, one can distinguish between the mechanism of damage.

Although more research needs to be done to develop the toxicity biosensor further, the use of this bioluminescent bacteria may be applicable to the toxicity monitoring system for biotreatment plants or in other areas, such as food, drinking water, and waste sample applications.

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REFERENCES

[1] Bulich, A. A. (1952) A practical and reliable method for monitoring the toxicity of aquatic samples. *Process Biochem.* 17: 45-47.

[2] Meighen, E. A. (1991) Molecular biology of bacterial bioluminescence. *Microbiol. Rev.* 55: 123-142.

[3] Hill, P. J., C. E. D. Rees, M. K. Winson, and G. S. A. B. Stewart (1993) The application of *lux* genes. *Biotechnol. Appl. Biochem.*17: 3-14.

[4] Stewart, G. S. A. B. and P. Williams (1992) *lux* genes and the application of bacterial bioluminescence. *J. Gen. Microbiol.* 138: 1289-1300.

[5] Gu, M. B., P. S. Dhurjati, T. Van Dyk, and R. A. LaRossa (1996) A Miniature Bioreactor for sensing toxicity using recombinant bioluminescent *Escherichia coli*. *Biotechnol. Prog.* 12: 393-397.

[6] Sameer R., M. B. Gu, K. Konstantinov, P. Dhurjati, T. K. van Dyk, and R. A. LaRossa (1996) Characterization of the stress response of a bioluminescent biosensor in batch and continuous cultures. *Biotechnol. Prog.* 12: 387-392.

[7] Van Dyk, T. K., W. R. Majarian, K. B. Konstantinov, R. M. Young, P. S. Dhurjati, and R. A. LaRossa (1994) Rapid and sensitive pollutant detection by induction of heat shock gene-bioluminescence gene fusions. *Appl. Environ. Microbiol.* 60: 1414-1420.

[8] Gu, M. B., G. C. Gil, and J. H. Kim (1999) A two-stage minibioreactor system for continuous toxicity monitoring. *Biosensors Bioelectronics* In press.

[9] Belkin, S., D. R. Smulski, S. Dadon, A. C. Vollmer, T. K. van Dyk, and R. A. LaRossa (1997) A panel of stress-responsive luminous bacteria for toxicity detection. *Water Research* 31: 3009-3016.

[10] Belkin, S. (1998) Stress-responsive luminous bacteria for toxicity and genotoxicity monitoring. p. 171-183. In: P. G. Wells, K. Lee, and C. Blaise (eds.) *Microscale Aquatic Toxicology-Advances, Techniques and Practice*. CRC Press. Boca Raton, FL.

[11] Henry, M. F. and J. E. Cronan (1992) A new mechanism of transcriptional regulation: release of an activator triggered by small molecular binding. *Cell* 70: 671-379.

[12] Cronan J. E. Jr. and O. R. Charles (1996) Biosynthesis of membrane lipids. p. 612-635 In: F. C. Neidhardt (ed.) *Escherichia coli and Salmonella Cellular and Molecular Biology*. Vol. 1. 2nd ed. ASM Press Washington D. C.

[13] Sikkema, J. J., A. M. Bont, and B. Poolman (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiol. Rev.* 59: 201-222.

[14] Siegele, D. A. and R. Kolter (1992) Life after log. *J. Bacteriol.* 174: 345-348.

[15] Nakamura, S., Y. Oda, T. Shimida, I. Oki, and K. Sugimoto (1987) SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. *Mut. Res.* 192: 239-246.

[16] Bruce, D. (1997) Regulation of bacterial oxidative stress genes. *Ann. Rev. Genet.* 25: 315-337.

[17] Budavary, S. (1989) *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals.* 11th ed. Merck & Co. Inc. Rahway, NJ.