박테리아세포칩을 이용한 환경독성 분석

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Bacterial Cell Arrays for Environmental Toxicity Analysis

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Introduction

Recombinant bioluminescent bacteria are increasingly receiving the attention as environmental biosensor due to its advantages, such as high sensitivity and selectivity, low cost, ease of use and short measurement time. When the bacterium experiences an environmental stress, a signal transduction cascade occurs in which certain promoters are induced and their proteins are expressed to adjust to the environmental stress. Recombinant bioluminescent bacteria have been developed that use these stress promoters for toxicity sensing and signal production. There are numerous stress genes in prokaryotic cells and fusions of their promoters with the *lux* genes results in new biosensing strains that emit light when stressed. Recombinant bioluminescent bacteria have been successfully used in detection of specific stress, including DNA damage, superoxide damage and membrane lipid damage. Some special compounds, causing toxic stress and becoming hot issues of society, also were analyzed their toxicities, for example endocrine disrupting chemicals, ionizing radiation and sixteen azo dyes. There are a lot of recombinant bioluminescent bacteria developed in the present and valuable information can be achieved in toxicity analysis through the use of these bacteria.

In this study, a whole cell-based array technology has been implemented to develop an environmental biosensor that used 20 different bioluminescent bacteria. Array technology allows the system to use much more bacteria in smaller size resulting in acquiring more information in a test. In addition, it is capable of be used in a simple and fast toxicity analysis like portable biosensor due to its small size and ease to handle.

Experimental

Twenty strains having different stress promoters were used to detect toxicity and bioluminescence change from each well was simultaneously measured by the use of highly sensitive cooled CCD

camera through obtaining images of the arrays and analyzing signal intensity using pixel density. Cell arrays have been fabricated on/in chip and 384 well plate by using immobilization technique (agar-LB mixture was used as immobilization matrix). Three chemicals that cause superoxide damage (paraquat), DNA damage (mitomycin C) or protein/membrane damage (salicylic acid) were tested with the cell arrays.

Results and Discussion

Bacteria cells used in this study were genetically engineered to contain a sensing-reporting construct by inserting the fusion of a reporter gene, *luxCDABE*, and specific stress promoter into the host bacteria. When the arrays exposed to toxicity, each well having different strains showed specific bioluminescent responses to toxicities according to promoter contained and, due to these specific responses, toxicity of samples can be characterized by the analysis of bioluminescent change from strains.

Two strains, EBSoxS(*soxS::luxCDABE*) and DS1(*sodA::luxCDABE*) showed high response to paraquat, superoxide stress causing agent. Bioluminescence generated by the result of the transcription of *soxS* was increased due to paraquat toxicity and cells clearly expressed detectable bioluminescent signals after 1 hr. Increase of bioluminescence was kept the pattern until 2 hr after exposing to paraquat, the highest light intensity was shown at 2 hr, and the light was rapidly dropped after 2 hr. A detectable bioluminescence from DS1 was observed only after 10min and the highest bioluminescent intensity was shown at 60min. It seems that paraquat caused a relatively low induction of *sodA* comparing to *soxS* but the expression time was much faster than that of *soxS* after treating with paraquat.

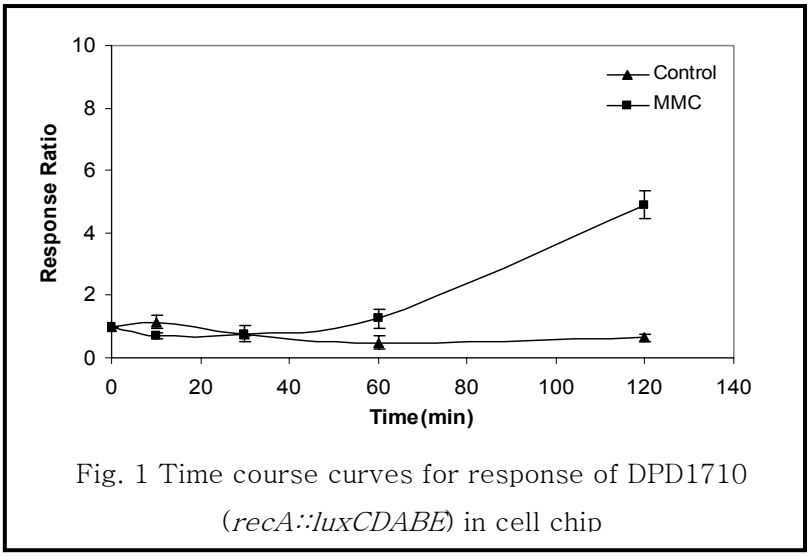

Fig. 1 Time course curves for response of DPD1710 (*recA::luxCDABE*) in cell chip

For toxicity test with Mitomycin C, bioluminescence couldn't be directly detected in images taken

by CCD camera due to low basal bioluminescence. However a bioluminescent increase caused by toxicity of MMC was clearly shown in analysis using pixel density. In figure 1, cell responses were expressed in relative pixel density unit (Pixel density at time t / Pixel density at time 0) and compared with control. As shown, mitomycin C induced the expression of *recA*, SOS-inducible gene, and although data was not shown here, TCA cycle involving gene *gltA* also showed about 2 fold inductions in bioluminescence, comparing with control. These shows that toxicity effects of mitomycin C is related with SOS regulon and TCA cycle in *E. coli*.

It was reported that salicylic acid caused an uncoupling of oxidative phosphorylation and induced transcription of heat-shock and membrane sensitive promoters. In this study, a promoter *grpE*, protein/heat shock stress promoter, and a membrane damage sensitive promoter *fabA* were induced in gene transcription to salicylic acid, resulting in bioluminescence increase.

It was shown in results of experiment conducted that all strains used in this study showed specific response to toxic chemicals according to their stress promoters. A bioluminescent response was observed only when strain met specific toxicity inducing the strain's promoter and no response to other toxicities.

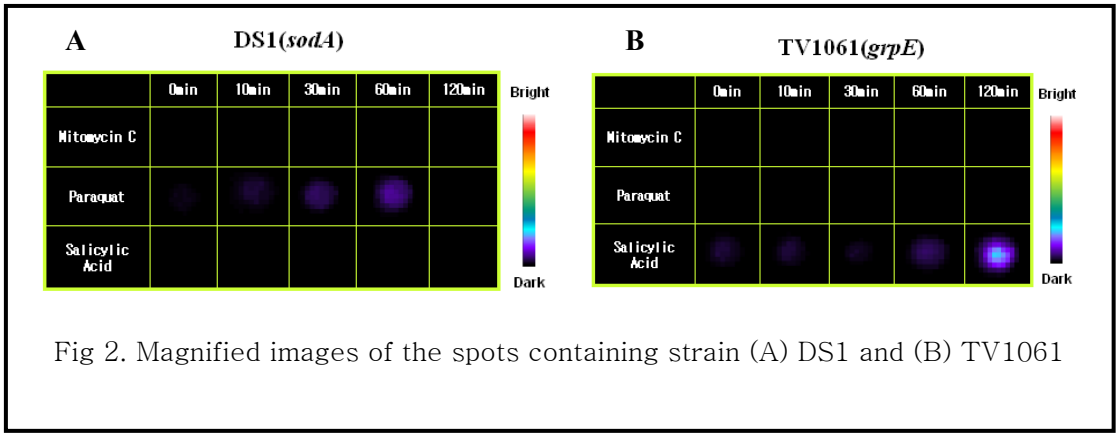

Fig 2. Magnified images of the spots containing strain (A) DS1 and (B) TV1061

Figure 2A show specific responses of DS1 to three chemicals. It showed strong response in bioluminescence to paraquat while no induction was shown to other samples. Similar response was also shown in EBSoxS. In contrast, strain TV1061 and DPD2540 showed transcriptional induction only by salicylic acid (Fig 2B). In the arrays, each spot carried different recombinant bacteria that can be induced by a specific stress. Therefore classification of toxicity is achieved in utilizing the cell arrays through the simultaneous changes seen in the bioluminescence of each spot.

Toxicities of three chemicals and distilled water tested in cell chips have been simultaneously tested in 384 well plate which is a set of four 96-well. Each chemical was injected into each 96-well and bioluminescence change from each well was directly compared with control, distilled water. For bioluminescent responses, strains were responded to toxic chemicals according to their stress promoters as shown in the cell chips but lower basal bioluminescence and more stable induction were

shown in 384 well plate compared to cell chip. It is remarkable that bioluminescent responses to salicylic acid were enhanced in stability and reactivity. Strain Kan3 and DO2 didn't respond to salicylic acid in cell chip but it responded in 384 well plat to salicylic acid. As well the bioluminescent induction of *fabA* was shown in images of 384 well plate taken over time after injection of salicylic acid due to toxicity.

Conclusions

Cell arrays have been successfully fabricated on/in chip and 384 well plate by using immobilization technique and twenty strains having different stress promoters. Bioluminescent Responses of 20 strains to toxicity were simultaneously analyzed by using sensitive cooled CCD camera through obtaining images of the arrays and analyzing signal intensity using pixel density. When the arrays exposed to toxicity, each well having different strains showed specific bioluminescent responses to toxicities according to promoter contained. Responses of the arrays to toxicity can be measured within 2 hour although response time was different in each chemical test depending on toxic properties of chemical tested and promoter.

These arrays can be employed for high-throughput environmental toxicity analysis and used as portable and disposable environmental biosensors due to its small size, ease handling, simple analysis and low cost.

Reference

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