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Application of Toxicogenomic Analysis to the Monitoring of Environmental Toxicity Using Recombinant Bioluminescent Bacteria and Cultured Mammalian Cells

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Abstract

Recombinant bioluminescent bacteria and cultured human cells were applied for toxicogenomic analysis of environmentally hazardous chemicals. Recombinant bioluminescent biosensing cells were used to detect and classify the toxicity caused by various chemicals. Classification of toxicity was realized based upon the chemicals' mode of action using DNA-, oxidative-, protein, and membrane-damage sensitive strains. As well, a simple double-layered cell culture system using Caco-2 cells and Hep G2 cells, which mimic the metabolic processes occurring in humans, such as adsorption through the small intestine and biotransformation in both the small intestine and the liver, was developed to investigate the toxicity of hazardous materials to humans. For a more in-depth analysis, a DNA microarray was used to study the transcriptional responses of Caco-2 and Hep G2 cells to benzo[a]pyrene.

Use of Recombinant Bioluminescent Bacteria for Toxicogenomic Analysis in Environmental Toxicity

Recombinant bioluminescent bacteria have been highlighted as toxicity biosensors because of their rapid response, low cost, and improved reproducibility. The recombinant bioluminescent bacteria uses stress promoters as toxicity mediators and bacterial luciferase as the reporter since it generates a bioluminescent output, which is correlated to the cells' response to sub-lethal toxic effects. One of the distinct merits of using stress promoters as switches representing toxicity is that different toxic materials can be classified based on their action by analyzing the substances' differential effects, such as protein damage, DNA damage, or oxidative damage, on a suite of bacterial strains containing various stress promoters [1-4].

In this study, various recombinant bioluminescent bacteria having different stress promoters were used to detect toxic chemicals. Initially, the response of these bacteria to a single chemical was determined for a wide variety of chemicals. The strains were found to be very specific to a certain group of chemicals, which were categorized based upon their toxicity to the cells. DPD2540 (*fabA::luxCDABE*), known to respond to membrane damage resulting in fatty-acid limitation, showed significant responses to known membrane-damaging agents, *i.e.*, phenol and cerulenin. DPD2794(*recA::luxCDABE*), a DNA-damage sensitive strain, responded to mutagenic chemicals, such as mitomycin C (MMC), while the oxidative- damage sensitive strain, DPD2511(*katG::luxCDABE*), showed a very specific response to hydrogen peroxide. Based on these results, each strain was proven to respond to a specific toxicity. In addition, the toxicity of many samples containing a single chemical or a mixture of chemicals in media, wastewater, or river water were successfully monitored and classified using the different strains, verifying their capabilities [3,4].

Furthermore, a more-in-depth study on phenolic toxicity was conducted and various phenolics classified using DPD2540, which was used to determine the extent to which phenolics result in the limitation of membrane fatty acids. Tested phenolics were found to be classifiable into two groups according to the bioluminescent response they elicited and their pKa. A dose-dependent bioluminescent response, due to fatty acid limitation, was seen for phenolics in Group I, which had pKa values of greater than 7 and exist mainly in the protonated form (HA), while no significant bioluminescent response was seen, compared to the control, for phenolics with pKa values lower than 7, which exist almost entirely as the dissociated form (A^-) in the media used in this study. A newly modified distribution model for phenolic compounds in the cellular membrane was proposed and used to predict the bioluminescent response induced by Group I phenolics and the cellular toxicity for both groups. The portion of the total undissociated phenol which comes into contact with the membrane system [HA]*, obtained with this model, showed good correlation with the various bioluminescent responses produced by Group I phenolics. It was also found that the distribution ratio between the medium and the cell membrane, K_I, calculated as well using the proposed model, is a representative parameter for the cellular toxicity of the phenolic compounds according to their substituted groups when compared with the conventional method of using the octanol-water partition coefficient, $\log K_{ow}$. A new parameter, the critical concentration, was also introduced and shown to be a good representative of the cellular toxicity, for Group I phenolics, to *Escherichia coli* cells [2].

Finally, for more practical use of these bacteria in the field, a freeze-drying methodology was introduced and a portable biosensor was developed [5-7]. A constitutive bioluminescent bacteria was used to monitor the cellular toxicity of chemicals by measuring the decrease in it's bioluminescence, while the specific toxicity was detected and monitored through the use of the inducible strains, DPD2540 (*fabA::luxCDABE*), TV1061 (*grpE::luxCDABE*), DPD2794 (*recA:: luxCDABE*), and DPD2511 (*katG::luxCDABE*). The toxicity of a sample could be determined 30 minutes after its addition to the freeze-dried strains. Therefore, these recombinant bioluminescent bacterial strains could be used as a primary screening system for further analysis of unknown samples.

Environmental Toxicity Evaluation Using Cultured Human Cells and Human DNA Microarray Technologies

The most common biological tools used to monitor the environmental safety problem rely on the experimental use of animals. In current society, however, there is a movement to decrease the number of animals needed for experimental studies. Therefore, a cellular model system using cultured human cells that mimic the metabolic activity of humans has been investigated as a possible alternative to the use of animal experiments [8]

In this study, a simple double-layered co-cultured system using Caco-2 cell and Hep G2 cell, which mimic metabolic processes occurring in humans, such as the absorption through the small intestine and biotransformation in both the small intestine and liver cells, was developed to investigate the toxicity of hazardous chemicals in humans [9]. To determine the feasibility of using this co-cultured system for toxicity-monitoring, benzo[a]pyrene (B[a]P) was tested as a model chemical. It was found that both Caco-2 and Hep G2 cells can metabolize B[a]P to toxic metabolites, possibly mediated by cytochrome P450 1A1/2 activity. However, in a double-layered co-culture system, the Hep G2 cells were protected from B[a]P toxicity through the action of Caco-2 cells in processes such as absorption, biotransformation, and the apical transport of B[a]P' metabolites. In comparing the viability reduction of Hep G2 cells caused by B[a]P, no significant reduction of viability was found in the co-culture system, although an approximately 60 % reduction in viability was observed in pure Hep G2 cultures. In addition, it was observed that Caco-2 cells transfer B[a]P and its toxic metabolites back to the apical side of the culture, evidenced by HPLC, thus decreasing the concentrations of toxic metabolites, such as 7,8-diol, encountered by the Hep G2 cells. As

such, a double-layered co-culture system can provide more accurate information regarding the toxic actions of the hazardous chemicals in humans than can a pure culture system, as it also gives the final toxicity as a result of many complicated phenomena, such as selective permeation in the small intestine and biotransformation in the small intestine and liver. Finally, DNA microarray technology was applied to provide a more-in-depth study of the transcriptional responses of Caco-2 and Hep G2 cells when exposed to B[a]P in both the pure and co-cultured systems.

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