Specific Responses of Freeze-Dried Recombinant Bioluminescent Bacteria to 16 Azo Dyes

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1. Introduction

Azo dyes are widely used in textiles, printed goods, cosmetics, drugs, food coloring, and other consumer goods. They are also used extensively in laboratories for either biological stainingor as pH indicators. The extent of their use within a society is related to the degree of industrialization[1,2] and it is possible that they may cause cancer. In fact, many azo dyes were shown to be genotoxic in short-termgenotoxicity tests and carcinogenic in tests with laboratories animals and microorganisms[1,2]. Azo dyes have for the most part been regarded as safe[2]. However, very little data has been published on the genetic and cellular toxicities of most dyes. Till now, many kinds of toxicityassays have been developed by necessity for rapid and sensitive methods. Researchers in Germany, France, and the Netherlands use the higher organisms such as fish or daphnia to detect the toxic substances discharged into the environments[3]. However, these methods were reported that they were less sensitive to hazard materials.

In this study, several recombinant bioluminescent bacteria were freeze-dried and usedto monitor and classify the toxicity of azo dyes since they were shown to be very reliable and stable biosensing tools. Of these, two constitutive bioluminescent bacteria, *Photobacterium phosphoreum* and GC2 (*lac::luxCDABE*)[4] were used to detect the cellular toxicity of the azo dyes, while four stress-inducible bioluminescent bacteria were used to provide information about the type of toxicity caused by the azo dyes dependent upon their toxic action and are DPD2794 (*recA::luxCDABE*), a DNA-damage sensitive strain, DPD2540 (*fabA::luxCDABE*), a membrane-damage sensitive strain, DPD2511 (*katG::luxCDABE*), an oxidative-damage sensitive strain and TV1061 (*grpE::luxCDABE*), a protein-damage sensitive strain[4].

2. Materials and Method

The recombinant inducible bioluminescent *E. coli* strains, DPD2794, DPD2540, DPD2511, and TV1061, all have RFM443 (*strR, galK2, lac74*) as a host harboring different plasmids[4]. These

recombinant plasmids contain a transcriptional fusion of the *E. coli* stress promoter to the *Vibrio fischeri luxCDABE* operon. Another recombinant bacterium, GC2, was also used and has the same host, RFM443, as above but was transformed with pLITE201, which has the *lac* promoter fused to the genes from *Photorhabdus luminescens* and confers resistance to ampicillin[4]. This strain produces bioluminescence constitutively.

All the azo dyes, *i.e.*, crystal violet, basic violet 1 and 2, direct blue 71, congo red, thiazol yellow G, orange II, acid green 25, reactive black 5 and 15, disperse yellow 3, and disperse orange 11, used in this study were purchased from the Sigma & Aldrich Chem. Co. The domestic production dyes, *i.e.*, navy blue, yellow, brown, blue and black, were obtained from Company A. Stock solutions were prepared using ethanol as a solvent for all 16 dyes and diluted into the media so that the final concentration of ethanol was below 0.1 %, a level that did not result in any significant stress response from the bioluminescent bacteria. The bioluminescence of the cells in the sample tubes was then monitored at set times using a luminometer (Turner Designs, TD-20e). The maximum BL ratio is definedin this study as the ratio of the maximum BL of the induced cells to the maximum BL of the control cells, exposed to ethanol alone. However, in the case of GC2 and *P. phosphoreum*, the constitutive strains, the relative bioluminescence (RBL) was defined as the ratio the BL of the induced cells to the BL of control cells 15 minutes post induction.

3. Results and Discussion

As shown in Fig. 1, crystal violet (basic violet 3) causes a serious amount of toxicity in the bacterial cells. Recombinant *E. coli*strain GC2 showed a dose dependent response, with a decrease in its bioluminescence, clearly demonstrating that crystal violet is toxic to bacteria (Fig. 1a). The effective concentration (EC20), which is defined as the concentration of chemical that produces a 20% decrease in the bioluminescence15 minutes after induction, was 0.1 mg/l for crystal violet. In addition, tests with *P. phosphoreum* gave similar results. However, the EC20 for crystal violet obtained with *P. phosphoreum*is higher than GC2, indicating that the recombinant bioluminescent bacterium is more sensitive than the naturally bioluminescent *P. phosphoreum*. All 16 azo dyes cause serious cellular toxicity within the two bioluminescent bacteria (data not shown). These dyes are classified into 6 differentgroups according to the staining methods used in the industrial applications. Within the basic dyes group, crystal violet was the most toxic as well as the mosttoxic of all 16 azo dyes, as based upon the EC20 values obtained with GC2 and *P. phosphoreum*. It was found that the level of cellular toxicity experienced due to the addition of these azo dyes, based upon their groups, decreased in the following order: basic dyes, direct dyes, disperse dyes, domestic dyes, acid dyes, and reactive dyes. The toxicity caused by each group of

dyes was found to be dependent on the water solubility. Five groups, all except the basic dyes, are also classified as being water-soluble, while only basic dyes are lipid soluble. Moller and Wallin reported that the insolubility of the dyes in water is the most important factor in the detection of their toxicological properties. This suggests that the solubility of azo dyes is an important factor in their uptake and bioavailability. In addition, the basic dyes were more toxic than other group because of their adsorption into cellular membrane.

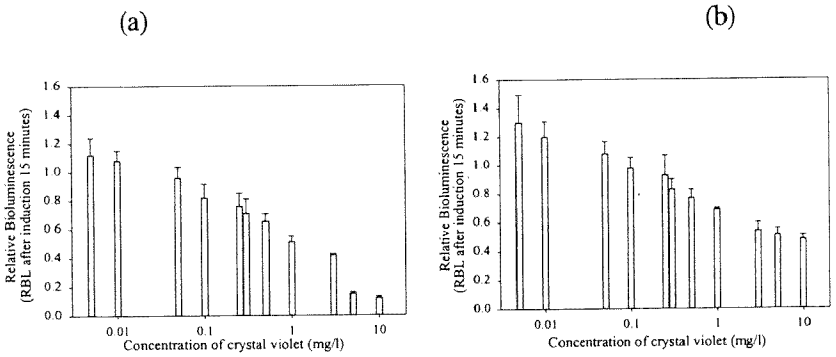

Figure 1. Dose-dependent response of the two constitutive bioluminescent strains to crystal violet. The RBL is the relative bioluminescence value at 15 minutes post induction. Response of (a) GC2 and (b) *Photobacterium phosphoreum*

4. Acknowledgement

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5. Reference

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