Environmental Biosensors Using Bioluminescent Bacteria

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Abstract

Environmental biosensors to assess the toxicity of environmental media such as water, soil, and atmosphere have been developed using various recombinant bioluminescent bacteria. These bacteria were constructed based on specific stress-responsive promoters in bacterial cells. They are thus activated by different groups of toxicity. For continuous monitoring of water toxicity, a multichannel system having different stress-responsive strains in each channel, and composed of two-stage mini-bioreactors, was successfully developed. Soil toxicity was assessed using a soil biosensor based upon immobilization of recombinant bioluminescent bacteria that worked with the addition of rhamnolipids biosurfactant. An example of phenanthrene toxicity is shown. For the assessment of gas toxicity, an immobilization technique has been set up to allow the biosensor to come in direct contact with the toxic gas in the sensing chamber. An example of benzene toxicity is shown. This mini review will show how the recombinant bioluminescent bacteria can be utilized as environmental biosensors. With further findings and developments of new non-specific stress promoters, the potency and extensiveness of the information that can be obtained using these environmental biosensors is immense.

Key words: biosensor, luminescent bacteria, soil, water, air, PAHs

63.1 Introduction

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 substrates, highly sensitive, and on-line measurability (Van Dyk et al. 1995; Vollmer et al. 1997; Gu and Choi 2001). Using bacterial bioluminescence as a reporter of toxicity has replaced the classical toxicity monitoring technology of using fish or Daphnia by cutting-edge technology. Fusion of bacterial stress promoters, which control the transcription of stress genes corresponding to heat-shock, DNA-, or oxidative-damaging stress (Van Dyk et al. 1995; Belkin et al. 1996; Vollmer et al. 1997) 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.
These recombinant bioluminescent bacteria have been used to develop toxicity biosensors in a continuous, portable, and in-situ measurement system for use in air, water, and soil environments (Gu et al. 1999, 2001a; Gu and Gil 2001; Gu and Chang 2001). All the data obtained from these toxicity biosensors within these environments were found to be repeatable and reproducible, and the minimal detectable level for the toxicity was found to be in the part per billion level for specific chemicals (Min et al. 1999; Choi and Gu 2001; Gu and Choi 2001; Gu et al. 2001c). Here, this short review will focus on how environmental biosensors and biomonitoring systems utilizing recombinant bioluminescent bacterial strains have been developed and implemented to detect the toxicity from air, water, and soil environments.

63.2 Experimental

63.2.1 Strains

Recombinant *Escherichia coli* DPD2794, containing a *recA::luxCDABE* fusion, as a model strain was used to monitor environmental damage to deoxyribonucleic acid (DNA), with mitomycin C as a model toxicant. And recombinant bacteria TV1061, containing a *grpE::luxCDABE* fusion, was used as another biosensor cell. This bacterial strain is responsive to toxicity due to protein-damaging agents. The DPD2540 strain, containing a *fabA::luxCDABE* fusion, was used as a biosensor cell responding to membrane-damaging agents. The GC2 strain, containing a *lac::luxCDABE* fusion, was used as a biosensor cell that is responsive to general toxicity causing cell death or luciferase inhibition.

63.2.2 System Development and Set-up Used in Those Biosensors

To minimize the operation cost and space for the set-up of this system, small bioreactors were fabricated with working volume of 10 or 20 ml. The mini-bioreactor has one side port, covered with 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 mini-bioreactor. 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 liquid volume/air volume/minute (l.v./v.m). Temperature was controlled by a water bath. After steady-state values of constant bioluminescence and cell density were obtained, the chemicals or test samples were injected into the second stage mini-bioreactor.

For the soil biosensor, the test reactor used is a 50 ml stainless steel cylinder having a water jacket to maintain a constant temperature using a heated circulation water bath (JEIO TECH, Korea). This reactor was filled with 25 ml of fresh Luria Bertani (LB) medium. Filtered air was supplied through a head port with a sparging tube while