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A whole-cell bioreporter assay for quantitative genotoxicity evaluation of environmental samples

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HIGHLIGHTS

- A bioreporter evaluates genotoxicity and bioavailability of environmental samples.
- The bioreporter is used in real world scenario for risk assessment.
- A gene regulation model is derived for SOS-based bioreporters.
- The gene regulation model enables quantitative genotoxicity assessment.

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ABSTRACT

Whole-cell bioreporters have emerged as promising tools for genotoxicity evaluation, due to their rapidity, cost-effectiveness, sensitivity and selectivity. In this study, a method for detecting genotoxicity in environmental samples was developed using the bioluminescent whole-cell bioreporter *Escherichia coli recA::luxCDABE*. To further test its performance in a real world scenario, the *E. coli* bioreporter was applied in two cases: i) soil samples collected from chromium(VI) contaminated sites; ii) crude oil contaminated seawater collected after the Jiaozhou Bay oil spill which occurred in 2013. The chromium(VI) contaminated soils were pretreated by water extraction, and directly exposed to the bioreporter in two phases: aqueous soil extraction (water phase) and soil supernatant (solid phase). The results indicated that both extractable and soil particle fixed chromium(VI) were bioavailable to the bioreporter, and the solid-phase contact bioreporter assay provided a more precise evaluation of soil genotoxicity. For crude oil contaminated seawater, the response of the bioreporter clearly illustrated the spatial and time change in genotoxicity surrounding the spill site, suggesting that the crude oil degradation process decreased the genotoxic risk to ecosystem. In addition, the performance of the bioreporter was simulated by a modified cross-regulation gene expression model, which quantitatively described the DNA damage response of the *E. coli* bioreporter. Accordingly, the bioluminescent response of the bioreporter was calculated as the mitomycin C equivalent, enabling quantitative comparison of genotoxicities between different environmental samples. This bioreporter assay provides a rapid and sensitive screening tool for direct genotoxicity assessment of environmental samples.

1. Introduction

As many anthropogenic contaminants are released into the environment, genotoxins are of great concern as they are potentially dangerous to the natural environment and human health (Shin, 2010). Chemical analysis can only quantify the total amount of chemicals within the samples, however suffering from high cost, and time-consuming and laborious operation. Moreover, chemical analysis does not directly provide integrated genotoxic effects or

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information on the bioavailability of various contaminants in complex environmental media (Shin et al., 2005; Jiang et al., 2016). A microbial whole-cell bioreporter typically combines a promoter–operator region in a bacteria host, which acts as the sensing device, with a reporter gene encoding for an easily detectable protein (Robbens et al., 2010). The unique feature of 'whole-cell' is that living microbial cells are used to obtain the bioavailable effects of a stimulus (Gu et al., 2004). Without the need of precise chemical characterization, whole-cell bioreporters are compact, portable, cost-effective and simple to use, providing an alternative approach for evaluating the general impacts of individual or mixed chemicals (Vollmer and Dyk, 2004; Nagata et al., 2010). Normally, whole-cell bioreporters are classified into two categories. One is responsive to specific toxicity pathways and induced in the presence of specific compounds or their analogues with similar structure, such as alkanes (e.g., alkane degradation pathway) (Sticher et al., 1997; Wang et al., 2016), naphthalene (e.g., naphthalene degradation pathway) (Neilson et al., 1999), polycyclic aromatic hydrocarbons (e.g., phenanthrene mineralization) (Tecon et al., 2009) and mercury (mercuric resistant regulatory pathway) (Rasmussen et al., 2000). The others can be induced by general toxicity pathways, including stressful conditions such as DNA damage (Vollmer et al., 1997; Min et al., 1999; Biran et al., 2009), membrane damage (Bechor et al., 2002) and oxidative damage (Lee and Gu, 2003). Bacterial SOS response is a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis are induced (Radman and Prakash, 1973; Little and Mount, 1982). RecA is essential in the SOS response of *E. coli*, responsible for DNA repair/maintenance via homologous recombination (Horii et al., 1980). Therefore, the *recA*-based whole-cell bioreporters are widely used for measuring general toxicity, capable of detecting not only the levels but also mechanisms of DNA damage (Sørensen et al., 2006; Ron, 2007), including DNA cross-linking and delayed DNA synthesis, alkylation and hydroxylation of DNA (Min and Gu, 2003; Chen et al., 2008). As most genotoxins are inducers of the SOS response (Quillardet et al., 1982), the *recA*-based bioreporter assay is introduced in genotoxicity assessment of environmental samples.

The use of living microorganisms as the sensing elements of a whole-cell bioreporter has several advantages over other assays such as enzymes, antibodies, or sub-cellular components based tests (Shin, 2010). Firstly, microorganisms can be genetically modified using mature protocols and are easily prepared by simple cultivation in relatively inexpensive media (Yu et al., 2006; Yagi, 2007). Secondly, a correlation between genotoxicity as measured by microbial bioassays and carcinogenicity in mammals has been found (Josephy et al., 1997), indicating whole-cell reporters can help in diagnosing the health risks of genotoxins to some extent. However, the microbial bioassay still suffers from a lack of eukaryotic metabolic enzyme systems (Lah et al., 2007), leading to uncertainties in extrapolating the genotoxic potency of one chemical from bacteria to eukaryotic cells, especially humans. Therefore, the whole-cell bioreporter assay cannot replace the role of direct measurement of carcinogenic effects in animals or humans, but still can be feasibly employed as a cost-effective and preliminary screening tool to assess ecotoxicity in environmental samples, particularly prior to well-established techniques (Alhadrami and Paton, 2013).

Although many whole-cell bioreporters are developed to sense the presence of specific chemicals or general toxicity, the majority of them are used still in laboratory proofs of concept (van der Meer and Belkin, 2010). In most cases, toxicities of chemicals in water samples or water extractions are evaluated by the bioreporter assay (Nagata et al., 2010; Zeinoddini et al., 2010; Axelrod et al., 2016). Recently, an *E. coli* bioreporter recApr–Luc2 was built to detect the genotoxicity of heavy metals in recycled ashes for livestock diets and evaluate their risks entering human food chain (Sanchezvicente et al., 2016). Nevertheless, the development of whole-cell bioreporters which are feasible in more complex environmental media (e.g., soils and seawater) is still challenging (van der Meer and Belkin, 2010; Michelini et al., 2013), as bioreporter sensitivity and chemical bioavailability are influenced by environmental variables (He et al., 2010; Jiang et al., 2015). Many attempts are made to overcome such barriers, and a limited number of bioreporters have been successfully applied in soils, seawater and groundwater (He et al., 2010; Zhang et al., 2012a; Yoon et al., 2016). Moreover, new techniques such as magnet-nanoparticles functionalization (Zhang et al., 2011; Jia et al., 2016) and microchip (Cortés-Salazar et al., 2013) are also developed to enhance bioreporter performance in complex environmental media.

The quantification of genotoxicity via a bioreporter assay has been conducted using two approaches. Taking the bioluminescent bioreporter as an example, the first approach compares the induced bioluminescent signals over time for different concentrations of target genotoxins to a negative control. Here, the parameter defined as the relative luminescent unit is derived, as the most commonly used quantitative method in bioreporter assays (Gu and Chang, 2001; Ore et al., 2010; Zeinoddini et al., 2010). By taking the end-point bioluminescence at time t as a function of the concentration series, the genotoxicity of unknown samples can therefore be quantified by interpolating their bioluminescent signals using the calibration curve. The other approach is to develop an analytical model for a whole-cell bioreporter to simulate their behaviors based on the quantitative SOS response of DNA damage inducible genes. Daniel et al. (2010) develops an analytical model of a whole-cell bioluminescent bioreporter, with an input signal (toxin concentration) and an output signal (bioluminescent light). The model is characterized by three measurable sets of parameters: the biosensor effective rate constant, the total number of emitted photons and the biosensor reaction order, verified for the three DNA damage inducible promoters, including *recA, katG* and *micF*. Recently, a gene cross-regulation model is developed to simulate the SOS response of the *A. baylyi* bioreporter (Zhang et al., 2012b). The model takes into consideration the dynamic variation in free RecA and single-stranded DNA (ssDNA)-bound RecA proteins, and the background expression of *luxCDABE* gene, correlating the input signal (genotoxin concentration) and output signal (bioluminescent light) with three empirical parameters: SOS response coefficient, genotoxicity coefficient and cytotoxicity coefficient. Although the mechanisms of *recA* gene induction and SOS response are similar in *E. coli* and *Acinetobacter baylyi* (Whitworth and Gregg-Jolly, 2000; Dolph et al., 2001; Hare et al., 2006), this mode has not been applied for *E. coli* bioreporter yet.

In the present study, a bioluminescent whole-cell bioreporter (Jiang et al., 2016) was employed to evaluate the genotoxicities and bioavailabilities of mitomycin C amended soils and seawater, which demonstrated the dose-effect relationships in both environments. Two case studies were further conducted on the bioreporter's response to chromium(VI) (Cr [VI]) contaminated soils and crude oil contaminated seawater. These two cases were chosen for the following reasons: i) Cr(VI) and crude oil are representatives of inorganic (e.g. heavy metals) and organic chemicals respectively and have high contamination levels in many regions of China and worldwide (Jacobs and Testa, 2005; Gao et al., 2015); ii) Cr(VI) and crude oil are known as genotoxins but with different mechanisms of DNA damage (Cohen et al., 1993; Mielżyńska et al., 2006), and it is therefore of great concern and importance to study the genotoxicity equivalent across different contaminants and