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A whole-cell bioreporter approach for the genotoxicity assessment of bioavailability of toxic compounds in contaminated soil in China

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ABSTRACT

A whole-cell bacterial bioreporter *Acinetobacter baylyi* strain ADP1_recA_lux that responds to genotoxins was employed to directly assess the adverse effects of the bioavailable fraction of mitomycin C (MMC), benzo[a]pyrene (BaP), chromium (VI) and lead (II) in amended soils and soil samples from two fragile areas in China without soil pre-treatment. The amended soils containing pollutants with the concentrations as low as 0.4 mg/kg MMC, 0.5 mg/kg BaP, 520 mg/kg Cr (VI) and 2072 mg/kg Pb (II) were found to be toxic. Soil particle-associated pollutants accounted for 86%, 100%, 29%, and 92% of the genotoxicity in the MMC, BaP, Cr (VI), and Pb (II) amended soil, respectively. The soils from contaminated sites were also valid to be genotoxic. The results suggest both free and soil particle-associated pollutants are bioavailable to soil organisms and a solid-phase contact bioreporter assay to soil contamination could provide a rapid screening tool for environmental risk assessment.

1. Introduction

Genotoxicity of contaminant in environment is one of the major public health concerns. Exposure to genotoxins may lead to cancer because they mutate or damage the genetic information within a cell. In china, vast lands have been contaminated by heavy metals and polycyclic aromatic hydrocarbons (PAHs) which form two large groups of compounds that are generally considered to be genotoxins (IARC, 2014). There are more than 4 million tons of untreated Cr slag throughout China and Cr contaminated soil has exceeded 12.5 million tons (China MoEP, 2005). 25,300 tons of total PAHs are generated annually from various sources in China (Wang et al., 2013) and the PAHs content reached 1 mg/g in the soil in the eastern and northern parts of China where some of the largest cities in the world are located (Yonghai et al., 2009). Cytotoxic drugs, which are designed to kill rapidly growing tumour cells, until recently, have not received much attention. Indeed, there are more than 100 cytotoxic drugs that are used routinely for chemotherapy in hospitals in developed countries, and the demand for chemotherapy treatment continues to increase by approximately 10% per year (Johnson et al., 2008; J.F. Zhang et al., 2013). However, cytotoxic drugs possess the potential to harm any growing organism that even a short-term exposure to a mixture of cytotoxic drugs at low concentration (ng/L) was reported to significantly inhibit human embryonic cell growth (Pomati et al., 2006) and their potential adverse effects on both human health and ecosystems cannot be ignored.

Most of the pollutants being released to environment are transported to soils and sediments where they are adsorbed or precipitated in the solid matrix, but the characterisation of the bioavailability and toxicity of those pollutants in soil was challenged due to the complexity of soils.

The application of bioassays has been applied to evaluate the biological effects of pollutants in soil. *In situ* bioassays in which employ native soil organisms such as protozoans, invertebrates, plants, and bacteria have been used (Alvarenga et al., 2013; Baek et al., 2004; Frische, 2003; Sverdrup et al., 2002, 2007, 2006; Xiao et al., 2006; Zhu et al., 2006). Bacterial whole-cell bioreporters (WCBs) are living microorganisms that are genetically engineered to produce signals in response to target chemicals or stress, enabling a rapid and sensitive detection of the bioavailable fractions in samples (Branco et al., 2013; Hagerberg et al., 2011; Ripp et al., 2011; Sharma et al., 2013; Tecon and van der Meer, 2008; van der Meer and Belkin, 2010). Since it was well accepted that

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only the soluble fraction of a pollutant was bioavailable and able to impose an effect on soil organisms (Boesten, 1993; Houx and Aben, 1993), previously contaminants were extracted from soils and then WCBs were applied to assess the bioavailability and toxicity of soil–solvent extractions (Dawson et al., 2008; Diplock et al., 2009; Flynn et al., 2002; Hagerberg et al., 2011; Hynninen and Virta, 2010; Kaekinen et al., 2011; Keane et al., 2008; Li et al., 2008; Liu et al., 2012; Paton et al., 2009; Patterson et al., 2004; B. Zhang, 2013; Zhang et al., 2012).

However, some recent studies have argued that the pollutants in non-aqueous phase were also able to exert a biological effect when applied WCBs to a soil-water suspension (also known as solid-phase contact assay). Ivask found that 20- to 115-fold more Cd, Pb, and Hg than them in water phase were bioavailable (Ivask et al., 2004, 2002). Maderova found that Cu in non-free ion speciation caused a bioreporter response (Maderova et al., 2011). Although WCBs have been applied to the detection of heavy metals, they have not been used to assess the geno-toxicity of bioavailable heavy metal in contaminated soil.

So far there were only a few studies to directly apply WCBs to soils for the assessment of contaminants including alkylbenzene sulphonate (Brandt et al., 2002), 2,4-dichlorophenoxyacetic acid (Hay et al., 2000), polychlorinated biphenyls (Liu et al., 2010), phenanthrene, and naphthalene (Ripp et al., 2000; Tecon et al., 2009), but the relationships between the bioavailable fraction and soluble phase fraction were not well addressed. Moreover, there is little information available regarding other commonly occurring organic pollutants, such as BaP, and emerging pollutants, such as cytotoxic drugs.

In this study, the *Acinetobacter baylyi* strain ADP1_recA_lux, was employed to assess the genotoxicity of soils contaminated with metals (chromium (VI) and lead (II)), a PAH compound (benzo[a] pyrene), and a chemotherapy drug (mitomycin C). The bioreporter ADP1_recA_lux was exposed to the contaminants in a solid-phase contact assay, and the response of the bioreporter was compared with soil–solvent extract analysis to identify the bioavailable proportion of the pollutants in soil. To examine the solid-phase contact genotoxicity assay to environment samples, ADP1_recA_lux was also exposed to six soil samples from an oil pumping field and Cr slag storage site in China and its performance was evaluated.

2. Material and methods

2.1. Bacterial strains and chemicals

Acinetobacter baylyi ADP1_recA_lux is a chromosomally based *Acinetobacter* bioreporter that fuses the promoter of bacterial SOS gene *rec*A with the bioluminescence reporter gene *luxCDABE* (Song et al., 2009). In the presence of genotoxins, ADP1_recA_lux can be induced to express bioluminescence (Song et al., 2009). ADPWH_*lux* is an *Acinetobacter* bioreporter induced by salicylate (Huang et al., 2005). In the presence of salicylate, it expresses the bioluminescence at high level, and in this study it serves as a light-off control for genotoxicity assay (see Supplementary material).

Unless otherwise stated, all chemicals were analytical-grade reagents (Sigma Aldrich, China). Luria–Bertani medium (LB) and LB medium supplemented with 10 μg/ml kanamycin (LBK10) was used for the cultivation of the bioreporters and in the induction studies.

One colony of ADP1_recA_lux was inoculated into 5 ml LBK10 liquid medium and incubated overnight (14–16 h) on a rotary shaker at 150 rpm and 30 °C. The cells were harvested by centrifugation at 3000 rpm for 5 min and re-suspended into 50 ml fresh LBK10 liquid medium.

2.2. Preparation of pollutant-amended soils

Reference soil GBW07412a (CRMCH, China) which is the brown soil collected from Liaoning Province in China was used to prepare the amended soil samples. The properties of the reference soil are listed in Table S1. The genotoxins MMC, BaP, Cr (VI) and Pb (II), were added to this standard soil. A series of solutions of MMC, potassium dichromate and lead acetate at different concentrations was prepared with deionised water; 0.1 ml of each solution was added to 0.1 g of soil in centrifuge tubes (glass tubes for MMC), and thoroughly mixed using a vortex. The mixture was then air dried for 48 h; the theoretical concentrations of MMC, Cr (VI) and Pb (II) were 0.004–4 mg/kg, 5.2–5200 mg/kg and 20.7–20720 mg/kg. To prepare the BaP-amended soil, BaP was dissolved in DMSO to make a concentration series ranging from 0.01 to 1000 mg/l. A 500-μl aliquot of the solution was then diluted in 20 ml acetone and added to 10 g of soil in a glass beaker. The mixtures were air dried for 48 h. All the amended soils were sieved through a 2-mm mesh and stored at 4 °C. To study the influence of the volatilisation and adsorption of MMC to the surface of the container during the amendment, a control experiment was conducted by following the amendment procedure but without adding soil to the container; the remaining MMC was re-dissolved with 0.1 ml of water.

2.3. Sampling of contaminated soil samples

The coordinates for sampling sites are listed in Table S2. Petroleum contaminated soils were sampled from Shengli Oil Field, Shandong Province, the second largest oil field in China. The major pumping area of the Shengli Oil Field is located in the wetland areas of the Yellow River Delta, which represents a highly valuable and diverse, yet vulnerable, ecosystem. Three samples were collected from two sites close an oil pump; and a background sample was collected one kilometre away from the oil pump.

Heavy metal contaminated soil was sampled at a chromium slag storage site of a former chromium salt manufacturer located in Shenyang City, Liaoning Province; 300,000 tons of Cr slag had been produced during past decades, and the Cr slag storage site covers an area of 18,000 m^2. The Cr slag storage site lies in a sensitive area where the nearest irrigation well is within one kilometre and the water source for Shenyang City is within ten kilometres. Two samples were collected from different layers of the top soil at the same location. The gravel and plant root residues were discarded, and the soil was air-dried. Two-millimetre meshes were used to sieve the samples. All the samples were stored at 4 °C.

2.4. Bioreporter ADP1_recA_lux response to geno-toxic compounds in water

The procedure of applying ADP1_recA_lux to samples was previously described (Song et al., 2009) with the following modification. Briefly, 198 μl of the cells and a 2-μl sample were transferred into each well of a 96-well microplate (black/clear bottom, Greiner, Germany) and mixed immediately for bioluminescence measurement. The 96-well microplate was incubated at 37 °C in a thermostat incubator with a shaking speed of 150 rpm, and the bioluminescence was measured every 30 min using a plate reader (Spectra M5, Molecular Device, USA).

For the samples containing BaP, the experiment was performed with the addition of human cytochrome P4501A1. The samples were mixed with 1.1 μl of 20× NADPH regeneration system solution A and 0.2 μl of 100× NADPH regeneration system solution B (Promega, UK) and 0.2 μl of P4501A1 (Sigma, UK) that simulate human response to convert BaP into a genotoxic metabolite; and 196.5 μl of bioreporter cells were then added to the sample.

2.5. Direct contact detection of DNA-damaging compounds in the soil matrix

To evaluate the interference effect of the soil matrix on bioluminescence detection (quenching or stimulation), the reference soils were mixed with ADP1_recA_lux and ADPWH_*lux* in different soil/medium proportions (0, 0.025, 0.05 and 0.5 g dry soil/ml). A 200-μl aliquot of the soil/cell mixture was pipetted into a 96-well plate, and the bioluminescence was measured as described above.

For heavy metal containing soil samples, 0.1 g soil was mixed with 10 ml of cells in 50-ml tubes and vortexed for 20 s. A 200-μl aliquot of the soil/cell mixture was pipetted into a 96-well plate, and the bioluminescence was measured.

For the soil containing MMC and BaP, 10 mg of soil was weighed and transferred to each well of a 96-well plate, and 200-μl aliquots of the cells were pipetted into a 96-well plate. For the BaP-amended soil, 1.5 μl of the P4501A1 and NADPH regeneration system mixture was added to the wells, and the samples were measured as described above. The samples from the Shengli Oil Field were assayed following the same procedure.

2.6. Chemical analysis of the samples

To extract the soil pollutants with water, 500 mg each of the MMC- and BaP-amended soils and 100 mg each of the heavy metal-amended soil was mixed with 10 ml of deionised water and kept shaking in a rotary shaker for 6 h. The supernatants were obtained by centrifugation at 8000 rpm for 10 min. The concentrations of MMC, BaP, Cr, and Pb in the supernatants were analysed by HPLC (Shimadz, JP), GC/MS (Waters, US) and ICP-AES (Thermo, US). To measure the total concentration of contaminants, the BaP-amended soil and petroleum contaminated soil were extracted using a Soxhlet extractor according to USEPA Method 3540C and then analysed by GC/MS. The exchangeable and acid-soluble portions of heavy metals in soil were assessed following the optimised BCR sequential extraction protocol (Sutherland, 2010). Each sample was mixed with 0.11 M acetic acid by end-over-end shaking (30 rpm) for 16 h at 22.5 °C. The supernatant was separated by centrifugation at 3000 × *g* for 20 min and analysed by ICP-AES.