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Evaluation of bioavailable arsenic and remediation performance using a whole-cell bioreporter

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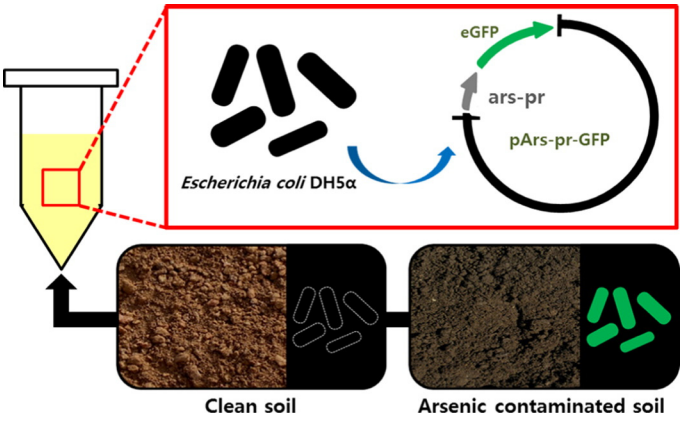
HIGHLIGHTS

- *E. coli*-based whole-cell bioreporter (WCB) was developed to detect arsenic in soil.
- The WCB expressed GFP under the control of an arsenic-responsive promoter.
- Soil samples artificially and naturally contaminated with arsenic were tested.
- The WCB was sensitive, detecting arsenic present in the parts per billion range.
- The WCB showed that soil washing reduced total but not bioavailable arsenic.

GRAPHICAL ABSTRACT



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ABSTRACT

The traditional method of evaluating the effects of soil contaminants on living organisms by measuring the total amount of contaminant has been largely inadequate, in part because testing contamination levels is hindered in real samples. Here we report a novel strategy for testing arsenic (As) bioavailability in soil samples by direct (in vivo) and indirect (in vitro) measurement using an *Escherichia coli*-based whole-cell bioreporter (WCB). The WCB was used to test As-amended Landwirtschaftliche Untersuchungs und Forschungsanstalt soils as well as field soils collected from a smelter area under remediation in order to evaluate the efficiency of bioavailable As removal. The percentage of bioavailable As in amended and field soils was 5.8% (range: 4.9%–7.6%) and 0.6% (0.08%–1.09%) of total As, respectively. In contaminated soils, total As was decreased, whereas bioavailable As was slightly increased after soil washing. These results emphasize the importance of considering ecotoxicological aspects of soil remediation; to this end, the WCB is a useful tool for evaluating the efficiency of soil remediation by assessing bioavailability along with the total amount of contaminant present.

1. Introduction

Arsenic (As) is a metalloid naturally present in the environment that is toxic at high concentrations to many organisms, including humans. Given its toxicity to insects, bacteria, and fungi, As is widely used in

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the agricultural industry; this can result in environmental contamination and poses health risks, including links to skin, bladder, and lung cancers, diabetes, and cardiovascular diseases (Abernathy et al., 2003; Smith et al., 1998; Tchounwou et al., 2004). It is therefore important to monitor environmental As levels and prevent its accumulation in the environment (Nachman et al., 2005; Rahman et al., 2004). In the case of soil contamination, remediation processes such as soil-washing are used to decrease As levels; soil remediation efficiency is then evaluated by assessing the total amount of As in the soil using extraction methods that employ acids, water, phosphate, or various chemicals (Alam et al., 2001; Turpeinen et al., 2003; Wenzel et al., 2001).

Although measurements of the total amount of contaminant in soil are used for risk assessment, they do not adequately reflect the actual hazards to living organisms, since heavy metals and metalloids are physically and chemically associated with soils and sediments. That is, the ecotoxicological effects of these substances on organisms cannot be readily distinguished from the physicochemical properties of soil (Davies, 1992; Sadiq, 1997). To address this problem, bacteria-based whole-cell bioreporters (WCBs) have been garnered interest for use in the determination of bioavailability (Belkin, 2003; van der Meer and Belkin, 2010). Although these have been widely used to monitor contaminant (e.g., heavy metal) levels in diverse environmental systems, their application to contaminated soils has been limited by the complexity of soil matrices. Since the water-soluble fraction of contaminants is generally considered as being bioavailable, WCBs have been used to determine the bioavailability of water-extracted contaminants (Hynninen and Virta, 2010; Liu et al., 2012). However, these are altered by the physicochemical properties of soil as well as the efficiency of extraction methods, which makes bioavailability measurements of the soluble fraction highly variable (Smith et al., 1999). Moreover, contaminants in the non-extracted water fraction are also biologically active and potentially harmful to living organisms (Ivask et al., 2004; Turpeinen et al., 2003). Therefore, a new strategy is needed to more accurately determine As bioavailability.

WCBs typically harbor reporter genes encoding fluorescent proteins, luciferase, or other enzymes that are under the control of contaminant-inducible regulatory elements and an As-inducible promoter (Gireesh-Babu and Chaudhari, 2012; Lewis et al., 2009; Robbens et al., 2010; Sorensen et al., 2006). The As-responsive transcriptional repressor *ArsR* suppresses reporter gene expression in the absence of As; when As is present, ArsR dissociates from the promoter, allowing the reporter gene to be expressed (Baumann and van der Meer, 2007; Diorio et al., 1995; San Francisco et al., 1990). Since the expression of the reporter gene is directly proportional to As concentration, the WCB system serves as a legitimate As sensor. Moreover, it is highly sensitive—detecting As levels in the parts per billion (ppb; μg/L) range—and highly selective for As over other heavy metals and metalloids (Loska et al., 2004; Robbens et al., 2010).

In this study, we investigated the applicability of WCB systems to quantify bioavailable As in the contaminated soils with minimizing the interferences of soil matrix and evaluated the efficiency of soil remediation in the aspect of bioavailability. In addition, we proposed a standardized WCB assay protocol for assessing bioavailable As efficiently in the contaminated soils. For this purpose, an *Escherichia coli* strain harboring an As-inducible promoter fused to enhanced green fluorescent protein (eGFP) was generated as an As-sensing WCB and used to determine the bioavailability of As in amended Landwirtschaftliche Untersuchungs und Forschungsanstalt (LUFA) soil and contaminated field soils collected from a smelter area before and after soil-washing. Contaminated soils were directly applied to the WCB and As bioavailability was analyzed by two different approaches—in vivo and in vitro assays which measure the fluorescent signal in *E. coli* cells and in soluble fraction, respectively—to minimize interference caused by the soil matrix. Based on our findings, we propose a standard protocol that avoids the interference of soil matrices and can be widely applied to the risk assessment of As in contaminated soils.

2. Materials and methods

2.1. Materials

E. coli DH5α was used as the host strain for plasmid construction and as the recipient for the pArs-GFP plasmid used to monitor As levels. The detailed procedure for the construction of pArs-GFP is described in the Supplementary information. Heavy metal(loid)s (As_2O_3, Na_2HAsO_4, $CdCl_2$, $K_2Cr_2O_7$, $CuCl_2 \cdot 2H_2O$, $HgCl_2$, $NiCl_2$, $PbCl_2$, and $ZnCl_2$) and anions (K_2SO_4, $KHPO_4$, KNO_3, NaCl, and Na_2CO_3) tested in present study were purchased from Sigma-Aldrich (St. Louis, MO, USA). LUFA soil was purchased from LUFA Speyer (Speyer, Germany) and used to prepare As-amended soil samples. Contaminated and remediated field soils were obtained from soil-washing project sites in Korea.

2.2. Preparation of soil and soil solutions

Artificially contaminated soil samples were obtained by adding arsenite [As(III)] and arsenate [As(V)] at final concentrations of 0, 1, 2, 5, and 10 μg/g to the vials containing 25 g of LUFA soil. The vials were maintained in the dark for 7 days before the WCB assay was performed. To prepare soil solution, contaminated soil was processed as follows using a previously described protocol (An, 2005; Turpeinen et al., 2003). Briefly, 100 mL distilled water were added to 25 g of soil sample, which was then mixed by rotation for 24 h. A solution was separated from the soil by passage through filter paper (8 μm pore size, Whatman #2); filtrates were passed a second time through a 0.45-μm cellulose filter (Advantec, Durham, NC, USA) and sterilized by autoclaving. Field soil samples before and after remediation were collected from three sites in Korea and were air dried before performing the WCB assay. Soil solution of contaminated field soil was prepared with the same protocol.

2.3. WCB assay

E. coli DH5α cells harboring pArs-GFP plasmid were grown overnight at 37 °C in Luria broth (LB) with 50 μg/mL ampicillin, and cells were added to 50 mL fresh LB and cultured overnight. When the optical density at 600 nm (OD_{600}) reached 0.4, different concentrations of heavy metal ion or soil sample were added to the cell cultures; 1 mL of sample was collected at different incubation times, and cells were harvested by centrifugation and resuspended in 1 mL of 50 mM Tris–HCl (pH 7.4) containing 160 mM KCl and analyzed using an FS-2 fluorospectrometer (Scinco, Seoul, Korea) and by fluorescence-activated cell sorting (FACS; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). GFP fluorescence intensity was represented as an induction coefficient, which was defined as (fluorescence intensity after addition of heavy metal)/(fluorescence intensity without addition of heavy metal). Protocols for fluorospectrometer measurements and FACS analysis are described in the Supplementary information.

2.4. Characterization of the WCB for As monitoring

To quantify bioavailable As levels in contaminated soils, the selectivity and sensitivity of the WCB for metal and metalloid ions were first characterized. WCB selectivity was tested by adding 1 μg/mL heavy metal or metalloid ions including Pb(II), Cr(VI), Cd(II), Cu(II), Hg(II), Zn(II), Ni(II) and As(III); 1 mL of cells was then harvested by centrifugation after 1 and 3 h of incubation at 37 °C. Cells were resuspended in 50 mM Tris–HCl buffer (pH 7.4) containing 160 mM KCl to avoid interference caused by LB medium. The sensitivity of WCBs for As was investigated by adding various concentrations of As(III) and As(V) ranging from 0 to 1 μg/mL to pre-incubated *E. coli* cells harboring pArs-GFP. In addition, anions known to be present in natural soils such as phosphate, sulfate, nitrate, carbonate, and chloride were tested to exclude their effects on the WCB assay.