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Growth and cesium uptake responses of *Phytolacca americana* Linn. and *Amaranthus cruentus* L. grown on cesium contaminated soil to elevated CO_2 or inoculation with a plant growth promoting rhizobacterium *Burkholderia* sp. D54, or in combination

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ABSTRACT

Growth and cesium uptake responses of plants to elevated CO_2 and microbial inoculation, alone or in combination, can be explored for clean-up of contaminated soils, and this induced phytoextraction may be better than the natural process. The present study used open-top chambers to investigate combined effects of *Burkholderia* sp. D54 inoculation and elevated CO_2 (860 $\mu L\,L^{-1}$) on growth and Cs uptake by *Phytolacca americana* and *Amaranthus cruentus* grown on soil spiked with various levels of Cs (0–1000 $mg\,kg^{-1}$). Elevated CO_2 and bacterial inoculation, alone or in combination, significantly increased biomass production with increased magnitude, ranging from 22% to 139% for *P. americana*, and 14% to 254% for *A. cruentus*. Total tissue Cs in both plants was significantly greater for bacterial inoculation treatment singly, and combined treatments of bacterial inoculation and elevated CO_2 than for the control treatment in most cases. Regardless of CO_2 concentrations and bacterial inoculation, *A. cruentus* had higher tissue Cs concentration, Cs transfer factors and concentration ratios than *P. americana*, but they had slightly different contents of antioxidant enzymes. It is concluded that combined effects of elevated CO_2 and microbial inoculation with regard to plant ability to grow and remove radionuclides from soil can be explored for CO_2- and microbe-assisted phytoextraction technology.

1. Introduction

Two ways have been attempted thus far to break through the application bottleneck of phytoextraction technology: enhancing the amount of plant biomass in the contaminated soils per unit area or increasing concentrations of metals and radionuclides in plants [1,2]. Inoculation of plants with microorganisms can increase plant biomass and uptake of metals and radionuclides in most cases [3–6]. For this reason, the interactions among metals/radionuclides, inoculated microbes and plants have attracted much attention because of the promise for practical application of microorganisms to metal/radionuclide removal directly from polluted soils or the possible transfer of accumulated metals/radionuclides to higher plants [5–7]. CO_2 enrichment in simplified greenhouses can promote plant growth and enhance uptake of heavy metals and radionuclides as well [2,8]. Elevated CO_2 not only increased above-ground biomass of the *Sorghum vulgare* × *Sorghum vulgare* var. *sudanense* hybrid and *Trifolium pratense* L. species, but also caused more accumulation of Cs [9]. Interestingly, elevated CO_2 induced higher rhizosphere soil microorganism populations of both *Sorghum* and *Trifolium* species, and this process may contribute to enhanced Cs accumulation by plants [9]. Conversely, it can be speculated that inoculation with external microorganisms in combination with elevated CO_2 may promote plant growth, enhance Cs uptake, and affect antioxidant defense systems in plants as well.

Despite recognition that elevated CO_2 or inoculation with plant growth promoting rhizobacteria (PGPR) has individual positive effects on plant growth and pollutant uptake [2,8–11], very little information is available about how plants grown on contaminated soils respond to elevated CO_2 and PGPR inoculation in combination. Also, few studies have been conducted to investigate combined effect of elevated CO_2 and bacterial inoculation on plant growth

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and uptake of radionuclides, especially in terms of developing CO_2- and microbe-assisted phytoextraction of contaminated soils. Such research is particularly important, as in reality, simultaneous application of multi-means is commonplace, as shown by microbe-associated phytoextraction in association with chemical soil manipulation. We hypothesized that the combination of elevated CO_2 and bacterial inoculation may positively affect *Phytolacca americana* and *Amaranthus cruentus* grown in Cs contaminated soil, consequently promoting plant growth, enhancing uptake of the pollutants, and altering antioxidant enzymes in plants. This hypothesis was based on two observations: one is that plant species have repeatedly been shown to have better growth and more uptakes of pollutants under elevated CO_2 [2,8–10]; the other is that plant growth is often improved under bacterial inoculation condition [1,3,4,11–13]. If this hypothesis is supported, the association of elevated CO_2 and bacterial inoculation can be developed into CO_2- and microbe-assisted phytoextraction of radiocesium from contaminated soils.

In the present study, we used open-top chambers to investigate the effects of inoculation with PGPR *Burkholderia* sp. D54, isolated from the heavily contaminated paddy field in Shangba village of the Dabaoshan mine in South China, and elevated CO_2 (760 $\mu L\,L^{-1}$) on plant growth, and their associated uptake of stable Cs by *P. americana* and *A. cruentus* grown on soil spiked with various levels of cesium (0, 200, 500 and 1000 mg Cs kg^{-1}). The main objective of the current work was to assess the possibility of using elevated CO_2 as a gas fertilizer in association with bacterial inoculation to promote the biomass production of the two tested plant species, and to increase the accumulation of Cs, making their combination more effective as phytoremediation agents. To our knowledge, this is the first report on the utilization of elevated CO_2 and bacterial inoculation, in combination, to induce phytoextraction of Cs from contaminated soils.

2. Materials and methods

2.1. Tested plant species

The seeds of *A. cruentus* L. were bought from National Crop Germplasm Conservation Centre of China and the seeds of *P. americana* Linn. were provided by Dr. S.G. Xue who first reported the finding of this plant species [14]. Both species were selected for this study due to their potential for phytoextraction of radiocesium contaminated soil [2,15,16]. *P. americana* Linn. was mistook as *Phytolacca acinosa* Roxb. due to their similarities in vegetative growth period without infructescence and inflorescence.

2.2. Bacterial isolation, identification, and inoculation

Pure culture of the bacterium strain D54 of the genus *Burkholderia*, isolated from the Dabao Shan mining area, Guangdong Province, Southeast China, was used in this study [11]. Ten grams of soil samples was put into 250 mL conical flasks, and 90 mL of sterile distilled water was added to each. The flasks were shaken at 150 rpm for 30 min at 30 °C in a rotary shaker (SKY-2102C, Sukun, China). One hundred microliters of the suspension were spread over the plates of modified SMN agar medium (mannitol, 1%; $(NH_4)_2SO_4$, 0.2%; peptone, 0.2%; K_2HPO_4, 0.05%; $MgSO_4$, 0.05%; $NaCl_2$, 0.01%; $FeSO_4$, 0.005%; $MnSO_4$, 0.005%; yeast extract, 0.05%; pH 7.0). The plates were incubated for 3 days at 30 °C. The single colonies that grew well on the modified SMN agar were picked up and streaked to other modified SMN agar plates supplemented with 0.5 g L^{-1} of tricalcium phosphate. After incubation for 3 days at 30 °C, the isolates that grew well on the plates were re-streaked three times to fresh modified SMN agar plate and stored on modified SMN agar slants. Genomic DNA extraction and the 16S rRNA gene PCR amplification of the isolate were carried out and identified following the procedures of Chun and Goodfellow [17]. The universal bacterial 16S rRNA gene primers (the forward primer P1: 5′-CGg gat ccA GAG TTT GAT CCT GGC TCA GAA CGA ACG CT-3′ and the reverse primer P6: 5′-CGg gat ccT ACG GCT ACC TTG TTA CGA CTT CAC CCC-3′) were used for the 16S rRNA gene PCR amplification of the isolate. The purified PCR product was directly sequenced by an automated DNA Sequencing System (ABI 3730XL). On the basis of the morphological, physiological and 16S rDNA gene sequence analysis, strain D54 was recognized as a species of the genus *Burkholderia* sp. (GenBank/EMBL/DDBJ accession no. HM467915) [11].

Actively growing cells were centrifuged at 10,000 rpm for 10 min and then washed with 0.85% (w/v) sterilized NaCl twice. The washed cells were re-suspended in sterilized de-ionized water to a final concentration of approximately 10^8 colony-forming units (cfu) mL^{-1} and used for plant inoculation.

Table 1
Physical and chemical characteristics of the soil used in this study.

	Values
Total N (g kg^{-1})	0.96
Total P (g kg^{-1})	0.51
Total K (g kg^{-1})	19.32
Available K (mg kg^{-1})	95.67
Cs concentration in soil (μg kg^{-1})	7.79
Organic matter (g kg^{-1})	16.25
CEC (cmol kg^{-1})	18.32
pH (H_2O)	5.52

2.3. The tested soil and its preparation for this study

The soil used in this study was collected from a long-term experimental rice field at Shenyang Agricultural University, Liaoning Province. The physical and chemical properties of the soil and background Cs concentration are shown in Table 1. The methods for soil property determination and elemental analysis follow Tang et al. [8]; a brief summary follows. The pH of soil was determined by a glass electrode (soil:water ratio, 1:2.5), organic matter by the potassium dichromate oxidation-heat method, total nitrogen by a semi-micro-Kjeldahl method, total phosphorus by the $HClO_4$–H_2SO_4 digestion ammonium molybdate ascorbic acid method, total potassium by $HCl + HNO_3 + HClO_4$ (3:1:1, v/v/v) digestion followed by Atomic Absorption Spectrometer (AAS) and a graphite tube equipped with an automatic sampler (ZEEnit 700, Analytikjena, Germany), and soil texture by the hydrometer method. The soil was digested overnight in 2 mL of concentrated HNO_3 at 120 °C, on a hot plate, and then was dissolved in 5 mL of HNO_3–HCl_4 (1:1, v/v) mixed acid and digested at 220 °C overnight for background Cs content analysis with the AAS. The soil is a sandy, silty loam (FAO classification) with high levels of organic matter (16.25%) and a pH of 5.52.

Fresh soil was drained through a 3-mm sieve and kept in darkness before use. Specified amounts of Cs ($CsCl_2$) in the form of dissolved solution were added and thoroughly mixed into the soil to give three levels: 200, 500 and 1000 mg Cs kg^{-1}. No Cs spiked soil was used as a control. The soil was kept in darkness for 75 days to allow equilibration of the substrate. The balanced soil was then transferred into 192 plastic pots (10 cm diameter and 12 cm height), each containing 1.0 kg dry weight soil. A suitable size plastic saucer was placed under the bottom of the filled pots to avoid leaching of soluble ions. Before planting, pots were fertilized with 200 mg kg^{-1} of N, 100 mg kg^{-1} of P, and 200 mg kg^{-1} of K, watered to maximum water holding capacity (WHC_{max}), and subsequently allowed to equilibrate for two more weeks. The pots