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Increased biomass and reduced heavy metal accumulation of edible tissues of vegetable crops in the presence of plant growth-promoting *Neorhizobium huautlense* T1-17 and biochar

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ABSTRACT

The effects of a plant growth-promoting *Neorhizobium huautlense* T1-17, biochar, and their combination on the biomass and Cd and Pb accumulation of Chinese cabbages and radishes and the mechanisms involved were characterized. T1-17 increased the biomass and reduced the above-ground tissue (Chinese cabbages) or root (radishes) Cd and Pb contents of the seedlings compared to the control. T1-17 and biochar + T1-17 significantly increased the edible tissue biomass (ranging from 56% to 112%) of the two vegetables compared to the control. T1-17, biochar, and their combination significantly reduced the edible tissue Cd and Pb contents (ranging from 46% to 86%) and total Cd and Pb uptake (ranging from 16% to 78%) of the two vegetables compared to the controls. Biochar + T1-17 had higher ability to increase the biomass and decrease the edible tissue Cd (Chinese cabbages) and Pb (radishes) contents than T1-17 or biochar. Furthermore, T1-17, biochar, and their combination significantly decreased the water-soluble (ranging from 32% to 88%) and DTPA-extractable (ranging from 14% to 51%) Cd and Pb contents in the rhizosphere soils compared to the controls. Notably, biochar + T1-17 had higher ability to decrease the water-soluble Cd and Pb contents of the rhizosphere soils than T1-17 or biochar. T1-17 and biochar + T1-17 significantly increased the ratios of small soil aggregate particles (<0.25 mm) of the rhizosphere soils of the two vegetables and negative correlation between the ratios of small soil aggregate particles and the DTPA-extractable Cd and Pb contents was observed. Furthermore, T1-17 and biochar + T1-17 significantly increased the ratio of IAA-producing bacteria in the rhizosphere soils of the two vegetables. The results showed the synergistic effects of T1-17 and biochar on the increased edible tissue biomass and decreased available Cd and Pb in the soils and Cd and Pb uptake of the vegetables. The results also suggested that T1-17 and biochar + T1-17 increased the edible tissue growth and reduced the edible tissue Cd and Pb uptake of the vegetables through increasing the proportions of plant growth-promoting bacteria and small soil aggregates in the rhizosphere soils.

1. Introduction

Atmospheric deposition and anthropogenic activities such as transportation emissions, fertilizer and pesticide use, wastewater irrigation, and mining activities are the major sources of heavy metal enrichment in soils (Nicholson et al., 2003; Ok et al., 2011; Fellet et al., 2014; Wagner and Kaupenjohann, 2014). Contamination of agricultural soils by heavy metals including Cd and Pb is a common problem on soils. Heavy metals in soils can be taken up by food crops and therefore they pose significant threats to human health and food safety (Babu et al., 2013). Numerous cases of heavy metal uptake by agricultural crops in polluted soils have also been reported (Chen et al., 1997; Keller et al., 2002). Vegetables are important component of human dietary system around the world and therefore their quality is closely related to human health. Many vegetables crops are still cultivated in the large-scale slightly and moderately heavy metal contaminated soils (Hao et al., 2011). Vegetables growing on heavy metal-contaminated soils may take up and accumulate heavy metals and cause health problems when consumed by humans and animals (Wang et al., 2004; Fu et al., 2008). Thus, developing efficient techniques to reduce heavy metal accumulation of vegetable crops in metal-contaminated soils is urgently and imperatively needed (Hlihor and Gavrilescu, 2009; Waqas et al.,

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2014). In situ metal stabilization or phytostabilization is regarded as a promising cost-effective and practical method for the reduced transfer of heavy metals to groundwater and soil ecosystems and the safe production of vegetables in the heavy metal-contaminated soils (Ma et al., 2011; Marques et al., 2013; Beesley et al., 2013; Ahmad et al., 2014). Phytostabilization is usually aided by the in situ application of soil amendments that are effective both in the immobilization of metals and the supply of material conditions that promote plant growth and stimulate ecological restoration (Bolan et al., 2011; Houben et al., 2012). In recent years, biochar and plant growth-promoting bacteria have been used for reducing the availability of heavy metals in soils and heavy metal accumulation of plants (Ma et al., 2011; Udeigwe et al., 2011; Marques et al., 2013; Waqas et al., 2014; Puga et al., 2015; Venegas et al., 2015). Biochars have been shown to adsorb heavy metals and decrease their bioavailability to plants and prevent uptake and food chain transfer (Méndez et al., 2012; Puga et al., 2015; Qiao et al., 2015). Recently, several studies have suggested that biochar can be an amendment for immobilization of heavy metals in contaminated soils (Namgay et al., 2010; Beesley and Marmiroli, 2011; Nielsen et al., 2014; Puga et al., 2015). Rajkumar et al. (2013) showed that plant growth promoting *Bacillus megaterium* SR28C stimulated the biomass of *Brassica juncea*, *Luffa cylindrica* and *Sorghum halepense* grown in both Ni contaminated and non-contaminated soils and reduced Ni uptake and translocation to the plants. Chatterjee et al. (2009) also reported that the inoculation of *Cellulosimicrobium cellulans* decreased Cr uptake in green chilli plants. Similarly, Vivas et al. (2006) found that the inoculation of *Trifolium repens* with Zn binding bacteria *Brevibacillus* sp. BeI decreased the concentration of Zn in shoot tissues compared with respective uninoculated controls. Moreover, the metal resistant bacteria play a great role in the growth and establishment of plants on the contaminated soils through producing plant growth beneficial metabolites including siderophores, indole-3-acetic acid (IAA) and 1-aminocyclopropane-1-carboxylate (ACC) deaminase and solubilizing phosphate (P) (Sun et al., 2010; Ma et al., 2011). Although biochar and bacteria have been reported to be used for heavy metal immobilization in soil and reduced metal uptake of plants in the heavy metal-contaminated soils, little is known about the synergistic effects of biochar and plant growth-promoting bacteria on the growth and heavy metal accumulation of crops in the heavy metal-contaminated soils (Wang et al., 2014; Touceda-González et al., 2015). Furthermore, no study has been carried on Chinese cabbage and radish growth and edible tissue Cd and Pb uptake and the mechanisms involved in the presence of plant growth-promoting bacteria and biochar. Bacteria producing indole acetic acid, siderophores, 1-aminocyclopropane-1-carboxylate deaminase and arginine decarboxylase are capable of stimulating plant growth and increasing heavy metal resistance of plants (Glick et al., 2007; Sun et al., 2010). Knowledge on the effects of biochar, metal-resistant and plant growth-promoting bacteria and their combination on the growth and heavy metal accumulation of vegetables is an important prerequisite for the better understanding of their interactions with the vegetables for the development of efficient and safe production of vegetables in the heavy metal-contaminated soils.

The objectives of this study were to analyze the effects of *Neorhizobium huautlense* T1-17, biochar, and their combination on the edible tissue growth and Cd and Pb accumulation of Chinese cabbages and radishes in the heavy metal-contaminated soils. The effects of T1-17, biochar, and their combination on the available Cd and Pb contents and the ratios of IAA-producing bacteria and soil aggregate particle sizes in the soils were also characterized, hoping to gain a more thorough understanding of the basis of the growth and Cd and Pb accumulation in these vegetables.

2. Materials and methods

2.1. Soil, bacterium and vegetable crops

The heavy metal-contaminated agricultural soils (Alfisol) were collected from the surroundings of a Pb/Zn mine wastelands located at Qixia, Nanjing (China) (118°57′E, 32°9′N). The soil analyses were as follows: soil pH was measured with a pH meter (PHS-3CT) after equilibrating 5 g of dry soils with 10 mL of deionized water for 30 min. Organic matter content, available N, P, K, cation exchange capacity (CEC), water-soluble and DTPA-extractable Cd and Pb contents were determined following the methods described in the Physical Chemical Analysis of soils (SSICA, 1980). Soil total Cu, Cd, Pb, and Zn were extracted with HF–$HClO_4$ (SSICA, 1980). The above Cu, Cd, Pb, and Zn concentrations in the extracts were determined with ICP-OES (inductively coupled plasma-optical emission spectrometer) (Optima 2100 DV; PerkinElmer, USA). Strain T1-17 isolated from heavy metal-contaminated soil can produce IAA (366 mg L^{-1}), siderophore, and ACC deaminase and has high Cd (2.2 mM), Pb (4.8 mM), Cu (7.9 mM), and Zn (7.7 mM) resistance. Strain T1-17 was identified as *Neorhizobium huautlense* based on 16S rRNA gene sequence (99%). The seeds of Chinese cabbage (*Brassica rapa* L. ssp. Chinensis L.) and radish (*Raphanus sativus* L. Var. Radculus pers) were purchased from the seed station of Jiangsu Academy of Agricultural Sciences. Chinese cabbage and radish belong to leaf and root vegetables respectively and are common planted vegetables in China.

2.2. Seedling growth and metal accumulation of the vegetables

The impacts of strain T1-17 on the seedling growth and Cd and Pb accumulation of Chinese cabbages and radishes were evaluated in plastic cups filled with 200 g of the metal-contaminated soils. Some properties of the soils were: pH (1:2 w/v water) 6.49; organic matter, 21.4 g kg^{-1}; total N, 1.78 g kg^{-1}; total P, 1.24 g kg^{-1}; CEC, 15.8 cmol kg^{-1}, total Cd, 5.8 mg kg^{-1}, total Pb, 876 mg kg^{-1}, total Zn, 219 mg kg^{-1}, total Cu, 49.6 mg kg^{-1}. The seeds of the vegetables were surface-sterilized with 10% H_2O_2 for 15 min and washed with sterile water. Ten surface-sterilized seeds were placed in each cup at a 1.0 cm depth. After germination, four seedlings were kept each cup. Strain T1-17 was grown in liquid LB medium for 20 h at 28 °C, centrifuged, washed, and resuspended to 1×10^8 cells mL^{-1} in sterile distilled water. Bacterial suspension (5 mL cup^{-1}) was sprayed on the soil surface 10 d after seedling emergence. A dead bacterial (autoclaved at 121 °C for 30 min) inoculated sample was prepared as a control. Triplicate cups were used for each treatment and placed in a greenhouse. The average temperature of the greenhouse ranged from 20.5 °C to 28.4 °C, the relative humidity was 68.5%, and an average photoperiod was 10 h per day. The plants were watered as required and harvested 45 days after inoculation. Roots and above-ground tissues were separated and washed, first in several changes of 0.01 M EDTA and then in distilled water to remove any nonspecifically bound Cd and Pb. Roots and above-ground tissues were oven-dried for 30 min at 105 °C, then at 55 °C, until they reached constant weights. The oven-dried samples were ground using a stainless steel mill (FZ102, Tianjing, China) to 0.5 mm for analysis. Subsamples of root (200 mg) and above-ground tissue (200 mg) samples were then digested in a mixture of concentrated HNO_3 and $HClO_4$ (4:1, v/v). Cd and Pb concentrations in the samples were analyzed using ICP-OES.

2.3. Pot experiment

The impacts of strain T1-17, biochar, and their combination on the edible tissue growth and Cd and Pb accumulation of Chinese