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Manipulation of the rhizosphere bacterial community by biofertilizers is associated with mitigation of cadmium phytotoxicity

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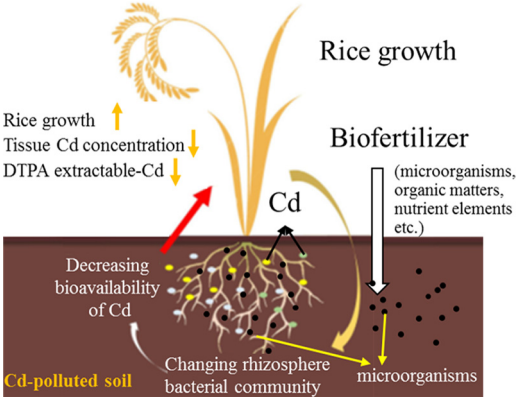
HIGHLIGHTS

- Biofertilizers were effective in mitigation of cadmium phytotoxicity.
- The rhizosphere bacterial community played critical roles in Cd stabilization.
- Effectiveness in mitigating Cd phytotoxicity was dependent on the type of biofertilizer applied.
- Soil physicochemical properties drove the structure of rhizosphere bacterial community.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to understand the effect of biofertilizers on cadmium (Cd)-induced phytotoxicity and the rhizosphere bacterial community. The crop specie rice (*Oryza sativa* L.) was planted in Cd-contaminated soils, and Illumina high-throughput sequencing was performed to investigate how the composition of the rhizosphere bacterial community responded to the addition of biofertilizers. Biofertilizers were effective in alleviating Cd phytotoxicity as indicated by the significant increase in plant biomass (up to 85.2% and 48.4% for roots and shoots, respectively) and decrease in tissue Cd concentration (up to 72.2% in roots) of rice receiving fertilizer treatments compared with the CK (no treatment). These positive effects were likely due to the increase in soil pH, which can be attributed primarily to Cd immobilization, and the promotion of beneficial taxa such as Proteobacteria, Bacteroidetes, Gemmatimonadetes, and Firmicutes. In addition, autoclaved biofertilizers tended to have similar beneficial effects and similar bacterial community alpha diversities as the original biofertilizer treatments. This suggests that the change in soil physicochemical properties by biofertilizer addition might drive the structure of rhizosphere bacterial community, and not the biofertilizer microbes themselves. In both the original and sterilized biofertilizer treatments, the effectiveness in mitigating of Cd phytotoxicity was found to be dependent on the type of biofertilizer applied. Comparatively, the biofertilizer denoted as DY was more effective in mitigating Cd phytotoxicity than others. These results demonstrate that biofertilizer addition

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could be a promising approach to immobilize soil Cd by manipulating the rhizosphere bacterial community, thus to facilitate plant growth.

1. Introduction

The rhizosphere is a narrow region of soil that adheres to plant roots, which plays a critical role in maintaining the balance of soil ecosystem, because complex biological and ecological processes, such as degradation of hydrocarbon compounds, the production of antibiotics, nutrients cycling, plant colonization or plant protection occur in this place (Marschner et al., 2003; Shen et al., 2015). Compared to bulk soil bacteria, rhizosphere bacteria live in more close association with plants, and display the advantages of promoting plant nutrient uptake, suppressing plant pathogens, or maintaining plant health (Muehe et al., 2015). Besides, rhizosphere bacteria contribute to the immobilization of metal ions and decrease their bioavailability through different mechanisms, such as extracellular complexation, precipitation, oxidation-reduction reactions or intracellular accumulation (Ahmad et al., 2008; Dennis et al., 2010; Kabeer et al., 2014; Deng et al., 2015).

Soil cadmium (Cd) contamination usually caused by mining, industrial or agricultural activities is considered one of the most severe environmental issues, because Cd is non-degradable and highly toxic, and has negative impacts on the human food chain and health (Bolan et al., 2015; Rizwan et al., 2016; Khan et al., 2017a). In recent years, the development of reliable, safe, environmentally friendly and cost-effective methods for controlling or reducing Cd contamination on agricultural land in China has aroused great interest (Wei et al., 2011; Tang et al., 2016). One potential method for counteracting Cd stress and increasing plant growth is the exogenous application of microbes. For example, the addition of *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Cupriavidus taiwanensis* and *Beauveria bassiana* to the soil was found to decrease Cd accumulation in rice (*Oryza sativa*) and increase plant growth and biomass under Cd stress; these beneficial effects were due to the formation of nontoxic insoluble cadmium sulfide (CdS) and adsorption by Cd-binding proteins (Siripornadulsil & Siripornadulsil, 2013; Suksabye et al., 2016). In another study, Moreira et al. (2014) reported that inoculation with plant-growth-promoting rhizobacteria (PGPR) increased maize growth and decreased Cd accumulation in shoots compared to the untreated control. Similar results were obtained by Sangthong et al. (2016) who found that application of Cd-resistant *Micrococcus* sp. TISTR2221 improved maize growth and reduced Cd accumulation in grains. Although the application of soil microorganisms has been widely reported to effectively improve plant health and stabilize soil Cd, the direct application of microorganisms onto fields without a suitable organic substrate is not expected to be stable, especially over the long term (Shen et al., 2015).

Biofertilizers, are usually formed by the solid-state fermentation of agro-industrial waste, they contain both microorganisms and primary nutrients or plant growth regulating substances (Chen et al., 2011). The application of biofertilizers into soil has been shown to improve the production of antibiotics and the biodegradation of soil organic matter, increase nutrient supply, enhance plant tolerance to environmental stress, therefore, biofertilizer has been adopted as a clean and efficient soil conditioner or amendment to improve the quality of soil by agriculturists and plant biologists (Gajdos et al., 2012; Bhardwaj et al., 2014; Shen et al., 2013). The combined benefits of fertilizers and bioagents may be expected to alleviate the effects of Cd toxicity on plant growth. Because the current hypothesis is that manipulation of the rhizosphere bacterial community could suppress Cd phytotoxicity, it is necessary to know how rhizosphere bacterial community composition and plant growth respond to biofertilizer application.

The objectives of this study were (1) to evaluate the effectiveness of biofertilizer addition on the mitigation of Cd phytotoxicity and (2) to determine the response of the rhizosphere bacterial community to biofertilizer amendment. Different biofertilizers, including autoclaved controls, were applied to Cd-polluted soil, and the biomass and Cd uptake of rice plants grown under each treatment were used to evaluate the efficiency of remediation. Illumina high-throughput sequencing of 16S rRNA was also applied to analyze the differences in the composition of the rhizosphere bacterial community after applying different biofertilizers. Based on the results of this study, a promising approach as applying biofertilizers into soil to immobilize soil Cd and optimize the composition of the rhizosphere bacterial community could be explored.

2. Materials and methods

2.1. Collection of samples

The naturally polluted soil samples (0–20 cm soil layer) used for laboratory experiments were collected from Hengyang (HY), Hunan province, China. The soil at this site was traditionally tilled for rice, and the climate and soil characteristics are shown in Table 1. Soil material was homogenized, air-dried and crushed. The soil was sieved (2-mm mesh size), and soil properties were determined according to standard methods. Soil pH was measured using a soil/water ratio of 1:5. Soil organic matter content was determined by combustion analysis (Marriott & Wander, 2016). Cation exchanging capacity (CEC) was measured using 1 mol/L ammonium chloride, pH 7.0 after pretreatment to remove the soluble salts (Oorts et al., 2007). Soil texture was analyzed as described by Tan (2005). Soil Cd concentration was determined using a PerkinElmer 1100B atomic absorption spectrometer. The biofertilizers used as amendments for remediation of Cd polluted soils were kindly supplied by the Center for Quality Supervision and Test for Microbial Fertilizers and Mushroom Spawn of the Ministry of Agriculture, Beijing, China. Biofertilizers were prepared using a solid fermentation method. Specifically, the first biofertilizer (DY) was prepared under aerobic conditions, using cattle manure supplemented with micronutrients and additives to stimulate fermentation. The second biofertilizer (AM) was produced under aerobic fermentation, and the organic substrates included oil rapeseed cakes and pig manure compost (1:1, w/w). The third fertilizer (HM) was prepared by fermenting bagasse and chaff at a ratio of 3:1 (w/w) using peat as the carrier in an aerobic environment. The biofertilizers were stored at 4 °C prior to use in pot experiments. The nutrient and bacterial compositions (determined as described in Section 2.3) of each biofertilizer are shown in Table 2.

2.2. Pot experiment

A 500 g soil sample was ground to pass through a 2 mm mesh sieve and placed into a plastic pot. Biofertilizers were blended into HY at a rate of 3%. To control for the effect of bacteria in biofertilizers on the remediation of Cd-polluted soils, biofertilizers were autoclaved in a steam pressurized vessel at 120 °C for 1 h and applied at the same ratio. Thus, the treatments in this study included (1) soils without any biofertilizer (CK); (2) soils with biofertilizer DY (DY); (3) soils with autoclaved DY (ADY); (4) soils with biofertilizer AM (AM); (5) soils with autoclaved AM (AAM); (6) soils with biofertilizer HM (HM); and (7) soils with autoclaved HM (AHM). NPK basal fertilizer containing 0.25 g Urea/kg soil, 0.15 g KH_2PO_4/kg soil and 0.04 g KCl/kg was first dissolved in deionized water and evenly mixed with the soil in each pot, and then all pots were incubated for 5 weeks with the moisture maintained at 75% of the