Environmental Pollution 241 (2018) 63–73

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Cultivar-specific response of bacterial community to cadmium contamination in the rhizosphere of rice (*Oryza sativa* L.)☆

Dandi Hou [a, b], Runze Wang [a], Xiaoyu Gao [a], Kai Wang [b], Zhi Lin [a], Jun Ge [a], Ting Liu [a], Shuai Wei [a], Weikang Chen [a], Ruohan Xie [a], Xiaoe Yang [a], Lingli Lu [a], Shengke Tian [a, *]

[a] MOE Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China
[b] School of Marine Sciences, Ningbo University, Ningbo 315211, China

ARTICLE INFO

Article history:
Received 15 January 2018
Received in revised form
23 April 2018
Accepted 25 April 2018

Keywords:
Cadmium
Rhizosphere
Bacterial community
Rice cultivar
Proteobacteria

ABSTRACT

Cadmium accumulation in rice grains is highly dependent on its bioavailability that affected by various physicochemical properties and microbiological processes of soil. The rhizospheric bacterial communities of rice grown in contaminated soils by means of rice cultivars highly or weakly accumulating Cd in grains (HA and LA, respectively) were investigated. HA roots absorbed 7.26- and 2.25-fold more Cd than did LA roots at low (0.44 mg kg^{-1}) and high (6.66 mg kg^{-1}) soil Cd levels, respectively. Regardless of Cd levels, Cd bioavailability in the rhizosphere of HA was significantly higher than that of LA. Planting of rice and elevated Cd levels both significantly decreased bacterial α-diversity and altered bacterial community structure, with noticeable differences between the rice cultivars. Taxa specifically enriched in the HA rhizosphere (phyla Bacteroidetes, Firmicutes, and Deltaproteobacteria) can directly or indirectly participate in metal activation, whereas the LA rhizosphere was highly colonized by plant growth–promoting taxa (phyla Alphaproteobacteria and Gammaproteobacteria). The results indicate a potential association of Cd uptake and accumulation with rhizosphere bacteria in rice grown on a contaminated soil, thus providing baseline data and a new perspective on the maintenance of rice security.

1. Introduction

Cadmium (Cd) contamination of soils has become a serious concern worldwidely due to its widespread distribution and high toxicity (Bertin and Averbeck, 2006). Cd is easily absorbed and accumulated by plants, thus constituting a serious threat to human health through Cd accumulation in the food chain (Park et al., 2011). As a global staple food, rice (*Oryza sativa* L.) is a significant source of Cd for humans through diet (WHO, 2011). The continually growing demand for rice and widespread contamination of soils with Cd urgently necessitates an understanding of how rice plants take up this metal from contaminated soils. Uptake of heavy metals by a plant is highly dependent on their bioavailability in the environment (Das and Maiti, 2008; Sanchez-Martin et al., 2007). It is therefore important to understand the main factors that regulate the transformation processes and bioavailability of heavy metals in paddy soils, particularly in the rhizosphere: a microenvironment where root–microbe–soil interactions actively take place (Hou et al., 2017; Yergeau et al., 2014; Abou-Shanab et al., 2003).

Heavy metal bioavailability in the rhizosphere is largely governed by metal speciation, soil type, and plant species as well as root-induced changes in physicochemical and biological soil properties (Dong et al., 2007; Martinez-Alcala et al., 2009). Because a dynamic habitat varies spatially and temporally (Yang and Crowley, 2000), the rhizosphere harbors one of the most complex and diverse plant-associated microbial communities (Lopes et al., 2016), which is directly or indirectly involved in metal mobilization and immobilization in the soil (Gadd, 2004). The processes caused by a rhizosphere microbe, such as chemical transformation, chelation, and protonation by the reduction of soil pH, the release of chelators, or redox changes, generally lead to mobilization of metals (Sessitsch et al., 2013), while the microbe-mediated bioprecipitation or biosorption decreases heavy-metal availability (Abdu et al., 2017; Zhuang et al., 2007). Moreover, some

☆ This paper has been recommended for acceptance by Joerg Rinklebe.

* Corresponding author. MOE Key Laboratory of Environmental Remediation and Ecological Health, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China.
E-mail address: tiansk@zju.edu.cn (S. Tian).

https://doi.org/10.1016/j.envpol.2018.04.121
0269-7491/

rhizosphere microorganisms may increase metal accumulation by stimulating plant growth (Muehe et al., 2015). A better understanding of how rhizosphere microorganisms are involved in a metal's bioavailability and its subsequent uptake by plant roots is therefore crucial to develop strategies to reduce health risks of rice grown on heavy-metal–contaminated soil.

Microbial community assembly in the rhizospheric zone is affected by abiotic and biotic factors (Philippot et al., 2013). Heavy metals in soil alter the microbial community that shapes the surroundings of the root surface, thus leading to a decrease in microbial biomass and a shift in the microbial community structure (Gao et al., 2010; Hoostal et al., 2008; Li et al., 2014) as well as the dominance of heavy–metal–resistant bacteria (Wood et al., 2016). Cadmium in soils alters soil microbial community structure, reduces microbial carbon use efficiency, and increases the microbial C:N ratio (Akmal et al., 2005). Nonetheless, most current studies on traits of the rhizobiome in heavy–metal–polluted soils are focused on the model plant *Arabidopsis thaliana* (Muehe et al., 2015), hyperaccumulating plants (Visioli et al., 2015), and metal-accumulating or metal-tolerant plants (Bell et al., 2015; Roman-Ponce et al., 2017). Little is known about the basic ecology of rhizosphere microbial communities associated with metal (especially Cd) uptake by staple rice crops in polluted soils.

Plants themselves provide a variety of carbon and energy sources, thereby influencing microbial populations in a plant-specific manner (Costa et al., 2006). Plant species and even cultivars within a species can form different microbial communities in the rhizosphere (Huang et al., 2014). This variation may be caused by the differences in root morphology as well as in the amount and type of rhizodeposits among individual plants (Philippot et al., 2013). Recently, a significant genotypic variation of rhizosphere microbial communities in rice plants was reported (Edwards et al., 2015). Nevertheless, the ecology of rhizosphere microbes in rice plants differs in Cd uptake and accumulation and their possible associations have not been reported. In this study, we planted two rice cultivars (differing in Cd accumulation in grains) on Cd contaminated soils to investigate the characteristics of their rhizosphere bacterial communities using 16S rRNA gene amplicon sequencing. We aimed to (i) compare bacterial rhizobiomes between the two rice cultivars in response to different Cd levels and (ii) understand the associations of the divergence in rhizosphere community composition with Cd uptake and accumulation in rice plants.

2. Materials and methods

2.1. Plant materials and soil characterization

Purplish clayed soil, a common paddy soil in the south of China, was collected from the surface layer (0–20 cm) of a paddy field located in Shaoxing, Zhejiang Province, China. Selected physicochemical properties of the soil are presented in Table S1. The soil was slightly polluted with Cd according to the Environmental Quality Standard for Soils of China (GB 15618-1995) and Environmental Quality Evaluation Standards for Farmland of Edible Agricultural Products of China (HJ/T 332-2006). Soil samples were air-dried, ground, and passed through a 2-mm sieve. In addition to the treatment with the initial low Cd level, a high-Cd treatment (6 mg kg^{-1}) was implemented by artificially adding Cd as a solution of $Cd(NO_3)_2$ to the pretreated soil. The Cd-spiked and unspiked soils were aged for 3 months at a moisture level of 60% of water-holding capacity prior to their use for pot experiments. At the end of the aging, the concentrations of Cd were 0.47 and 6.66 mg kg^{-1} respectively in the unspiked (low-Cd) and Cd-spiked (high-Cd) soils.

Seeds of two rice (*Oryza sativa* L.) cultivated varieties, highly accumulating Cd in the grains (Zhefu No. 7; hereafter: HA) and weakly accumulating Cd in the grains (Xiangzaoxian No. 45; hereafter: LA) were surface-sterilized with 70% ethanol for 1 min and 0.01 g mL^{-1} sodium hypochlorite for 5 min, rinsed thoroughly in deionized water, and germinated at 37 °C in the dark. After that, the rice seedlings were transplanted to a nutrient solution (Yang et al., 1994) for preculture for 2 weeks.

2.2. Plant culture and soil sampling

Two root bags (polyester mesh; 300 mesh pore size, 6 cm diameter, 20 cm height) were filled with the prepared soil (0.5 kg) and evenly distributed in each microcosm (20 cm height × 22 cm diameter). The remaining soil (4.5 kg) was used to fill spaces between the root bags. After 2-week preculture, uniform seedlings of the LA and HA cultivars were selected and each seedling was transplanted into each root bag. Treatments included (1) low-Cd soil without plants, (2) low-Cd soil planted with HA rice, (3) low-Cd soil planted with LA rice, (4) high-Cd soil without plants, (5) high-Cd soil planted with HA rice, and (6) high-Cd soil planted with LA rice. Three replicates with two plants each were prepared, and the treatments without planting were also repeated three times. All the pots were randomly arranged in a greenhouse with a light/dark period of 16/8 h, day/night temperatures of 30/25 °C, day/night relative humidity of 70%/85%, and photon flux density of 400 μmol m^{-2} s^{-1}.

Plants were harvested at maturity after 3 months of the pot experiment from September to November 2015. The soils and plants were removed from each pot, and the roots were removed from the soils in the root bags. The rhizosphere was sampled according to the methods described by Edwards et al. (2015). Briefly, we manually shook off the excess soil from the roots, leaving approximately 1 mm of soil still attached to the root system. The 1 mm of the adherent soil was washed off in 50 mL of phosphate-buffered saline (PBS) and kept as rhizospheric soil samples. Approximately 5 g of bulk soil was collected from the center of a soil layer between two plants, and unplanted samples were collected in the center of the pots without planting.

2.3. Analyses of soil properties and elemental concentrations

Soil properties and elemental concentrations were analyzed in soil samples from the rhizosphere, bulk zone, and unplanted microcosm, both at the beginning and at the end of the experiment. The metadata of the 22 soil environmental variables, including soil pH, total organic carbon (TOC), total or available nutrient elements and heavy metals are summarized in Table S2. To analyze the concentrations of total Cd and other elements, soil samples (0.1 g) were digested with 5 mL HNO_3, 1 mL $HClO_4$, and 1 mL HF at 180 °C for 10 h. Bio-available Cd and other elements were extracted with diethylene triamine pentaacetic acid (DTPA) extracting agent (0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine, pH 7.3). Soil samples were analyzed for DTPA-extractable heavy metals in a 1:2 soil:solution mixture, which was obtained after shaking for 2 h. Concentrations of elements in the digestive and extractive solutions were determined by inductively coupled plasma mass spectrometry (Agilent 7500a, USA). Sample replicates, reagent blanks, soil standard reference material (GBW07429, the National Research Center for Certified Reference Materials of China) were included in each batch of analysis to ensure the quality of analysis. The recovery of the standard for each element was 90–110%.

All the data are reported as mean ± standard deviation of three replicates. Significance of differences ($P < 0.05$) among treatments was evaluated by protected Fisher's least significant difference