Ecotoxicology and Environmental Safety 145 (2017) 111–118

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops

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ARTICLE INFO

Keywords:
Bacterial community structure
Cadmium
Phytoremediation
Bioenergy crops
MiSeq sequencing
Molecular ecological network analysis

ABSTRACT

Two energy crops (maize and soybean) were used in the remediation of cadmium-contaminated soils. These crops were used because they are fast growing, have a large biomass and are good sources for bioenergy production. The total accumulation of cadmium in maize and soybean plants was 393.01 and 263.24 μg pot^{-1}, respectively. The rhizosphere bacterial community composition was studied by MiSeq sequencing. Phylogenetic analysis was performed using 16S rRNA gene sequences. The rhizosphere bacteria were divided into 33 major phylogenetic groups according to phyla. The dominant phylogenetic groups included Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, and Bacteroidetes. Based on principal component analysis (PCA) and unweighted pair group with arithmetic mean (UPGMA) analysis, we found that the bacterial community was influenced by cadmium addition and bioenergy cropping. Three molecular ecological networks were constructed for the unplanted, soybean- and maize-planted bacterial communities grown in 50 mg kg^{-1} cadmium-contaminated soils. The results indicated that bioenergy cropping increased the complexity of the bacterial community network as evidenced by a higher total number of nodes, the average geodesic distance (GD), the modularity and a shorter geodesic distance. Proteobacteria and Acidobacteria were the keystone bacteria connecting different co-expressed operational taxonomic units (OTUs). The results showed that bioenergy cropping altered the topological roles of individual OTUs and keystone populations. This is the first study to reveal the effects of bioenergy cropping on microbial interactions in the phytoremediation of cadmium-contaminated soils by network reconstruction. This method can greatly enhance our understanding of the mechanisms of plant-microbe-metal interactions in metal-polluted ecosystems.

1. Introduction

Agriculture, mining and industry are major contributors to heavy metal soil contamination. Environmental degradation is prevalent in China, with approximately 20% of the arable land estimated to be contaminated with heavy metals (Teng et al., 2014; Zhao et al., 2015). This degradation is likely to have severe global economic and geopolitical consequences in the future. As a potential solution, phytoremediation has been found to be superior to existing conventional technologies used for heavy metal extraction from soils (Salt et al., 1995; Ali et al., 2013). Specifically, energy crop cultivation, as an alternative land-use strategy, is a significant component of biomass production and the ecological remediation of sites contaminated by heavy metals (Shi and Cai, 2009; Weyens et al., 2009; Gomes, 2012).

Important and complex symbioses exists between metal-tolerant and metal-accumulating plants and their rhizosphere microflora. Understanding these relationships might facilitate the development of phytoremediation technologies for removing heavy metals from contaminated soils (Rajkumar et al., 2012; Teng et al., 2015). Microorganisms coexist and interact within a complex system of networks, with both positive and negative feedback loops. Understanding these interactions is a central theme in microbial ecology (Faust et al., 2012;

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http://dx.doi.org/10.1016/j.ecoenv.2017.07.019
Received 31 March 2017; Received in revised form 6 July 2017; Accepted 10 July 2017
Available online 13 July 2017
0147-6513/

Deng et al., 2016). For example, Sheng et al. (2012) examined the rhizosphere bacterial communities associated with copper-contaminated soils of the important biofuel crop maize (*Zea mays*) using denaturing gradient gel electrophoresis. Network models can be used for depicting plant-microbe relationships; however, the development of these models lags far behind the advances in metagenomic technologies (e.g., sequencing and microarrays) (Faust et al., 2012; Deng et al., 2016). Conventional analytical techniques have failed to rival network analyses in revealing pivotal information on the interactions among organisms (Bahram et al., 2014; Banerjee et al., 2016), keystone microbial taxa (Lu et al., 2013; Banerjee et al., 2016), and changes in environmental factors (Zhou et al., 2011; Jiang et al., 2015; Tu et al., 2015; Creamer et al., 2016). In a recent study by Deng et al. (2016), functional molecular ecological networks were constructed to study the effect of emulsified vegetable oil (EVO) amendment on groundwater microbial communities. The results showed that EVO injections triggered bacterial competition. Next-generation sequencing (NGS) technologies, including the 454 and MiSeq platforms, have the potential to revolutionize environmental microbiology because they allow the resolution of these complex networks and the association of microbial communities with their associated niche functions (Zhou et al., 2010, 2011; Hahn et al., 2016).

We chose maize and soybean in the current study due to their rapid growth rates, large biomass and bioenergy utility (Shi and Cai, 2009; Sheng et al., 2012; Van Slycken et al., 2013; Pandey et al., 2016). The rhizosphere bacterial community composition was studied by MiSeq sequencing. This study represents the first network study of the impact of biofuel crops on microbial interactions in cadmium-contaminated soils, and it aims to enhance understanding of the mechanisms of plant-microbe interactions in these systems. The findings may contribute to the development of an effective bioenergy plant-microbe partnership for the phytoremediation of cadmium-contaminated soils.

2. Materials and methods

2.1. Pot experiments

Non-metal-contaminated soil samples were collected from cultivated soil in Nanyang, China (32°99′N and 112°47′E). The characteristics of the soil samples were as follows: yellow cinnamon soil; pH, 7.62; total P, 1.90 g kg^{-1}; available P, 18.27 mg kg^{-1}; total N, 1.31 g kg^{-1}; organic matter, 10.29 g kg^{-1}; cation exchange capacity, 13.2 cmol kg^{-1}. For the pot experiments, air-dried soil samples were thoroughly mixed and sieved (2 mm). The soils were treated with $CdSO_4{\cdot}8H_2O$ to achieve a cadmium concentration of 50 mg kg^{-1}, and the treated soils were left undisturbed for 15 days (for metal stabilization). In the pot experiments, plastic pots (diameter 20 cm and height 12 cm) were used, each containing 2.0 kg of soil with cadmium concentration of 0 or 50 mg kg^{-1}. Maize plants (*Zea mays*) variety Zhengdan-958 and soybean plants (*Glycine max*) Zhonghuang-57 were used in the phytoremediation experiment. In the pot experiment, there were a total of six treatments, with each treatment conducted in triplicate pots. The planted pots were maintained in the greenhouse of Nanyang Normal University from October to December 2015. The soil water content of the plants in the well-watered plots was maintained at 60–75%.

2.2. Energy crops growth and cadmium accumulation

After 60 days, the plants were harvested. The roots were immersed in EDTA (0.01 M) solution for 30 min and washed by deionized water several times to remove bound cadmium. The root lengths and the above-ground tissue lengths were measured. Plant tissues were oven-dried for 30 min at 105 °C and at 80 °C thereafter until achieving constant weight. The plant tissues were ground and digested with HNO_3/$HClO_4$ (4/1, v/v). The cadmium concentrations of root, stem and leaf tissues were analyzed by using an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Optima 2100 DV, Perkin Elmer, USA).

Tolerance index (TI), bioconcentration factor (BCF) and translocation factor (TF) values were used to estimate the crop potential for phytoremediation use. TI was expressed as the ratio of lengths or weights of the roots and the above-ground tissues in heavy metal-contaminated soil to those in control soil as follows (Ait Ali et al., 2002):

$$\mathrm{TI} = \frac{\text{Growth parameters of the plants grown in cadmium} - \text{contaminated soil}}{\text{Growth parameters of the plants grown in control soil}} \times 100$$

The TF of cadmium from the roots to the above-ground tissues was calculated as follows (Audet and Charest, 2007):

$$\mathrm{TF} = \frac{\text{cadmium accumulation in above} - \text{ground tissues}}{\text{cadmium accumulation in roots}}$$

BCF was expressed as the ratio of the heavy metal concentration in plant tissues to that in soil. Two BCF measures (roots and above-ground tissues) were calculated as follows (Audet and Charest, 2007):

$$\mathrm{BCF} = \frac{\text{cadmium concentration in roots or above} - \text{ground tissues}}{\text{cadmium concentration in soil}}$$

2.3. DNA extraction, PCR, and MiSeq sequencing

To collect rhizosphere samples, plants were carefully excavated, and the soil loosely attached to the root was removed. Genomic DNA was extracted from 0.5 g of fresh rhizosphere soil using the Fast DNA® SPIN for Soil Kit (MP Biochemicals, Solon, OH, USA) according to the manufacturer's instructions. The V3-V4 region of the bacterial 16S rRNA gene was amplified (95 °C for 3 min; followed by 27 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s; and a final extension at 72 °C for 10 min) using 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) with sample-identifying barcodes. The PCR assays were performed in 20 μL mixture containing 4 μL of 5 × FastPfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, 10 ng of template DNA, and Milli-Q water. PCR was performed in triplicate for each sample, and the products were purified using the AxyPrepDNA Gel Extraction Kit (Axygen, USA) and re-quantified with QuantiFluor™ ST (Promega, USA). The sequencing was performed by Shanghai Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) with an Illumina MiSeq PE300 platform.

2.4. Pyrosequencing data

The sequence data were processed using the Quantitative Insights Into Microbial Ecology (QIIME) Pipeline (Version 1.7.0 http://qiime.org/tutorials/tutorial.html). The operational taxonomic units (OTUs) at 97% similarity level were clustered using Usearch (version 7.1 http://drive5.com/uparse/). The OTU number of each sample was used to represent species richness. Rarefaction curves and Shannon-Wiener indices were generated, and the ACE, Shannon, and Chao1 estimators were calculated to compare the bacterial richness and diversity. Taxonomic classification at the phylum and genus levels was performed using the Ribosome Database Project (RDP) algorithm to classify the representative sequences of each OTU. A principle component analysis (PCA) was performed at a 3% dissimilarity level. The linear discriminate analysis (LDA) effect size (LEfSe) (http://huttenhower.sph.harvard.edu/lefse/), a statistical tool designed for the identification of biomarkers from metagenome data, was used to identify potential statistically significant taxa between different treatments (Segata et al., 2011).