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Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar

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ABSTRACT

Rhizosphere bacteria have significant contributions to crop health, productivity and carbon sequestration. As maize (*Zea mays*) is an important economic crop, its rhizosphere bacterial communities have been intensively investigated using various approaches. However, low-resolution profiling methods often make it difficult to understand the complicated rhizosphere bacterial communities and their dynamics. In this study, we analyzed growth-stage related dynamics of bacterial community structures in the rhizosphere of maize using the pyrosequencing method, which revealed an assembly of bacteria enriched in the rhizosphere. Our results revealed that the rhizosphere of maize was preferentially colonized by Proteobacteria, Bacteroidetes and Actinobacteria, and each bacterial phylum was represented by one or two dominating subsets of bacterial groups. Dominant genera enriched in the rhizosphere included *Massilia*, *Burkholderia*, *Ralstonia*, *Dyella*, *Chitinophaga* and *Sphingobium*. Rhizosphere bacterial community structures significantly changed through different growth stages at lower taxonomic ranks (family, genus and OTU levels). Genera *Massilia*, *Flavobacterium*, *Arenimonas* and *Ohtaekwangia* were relatively abundant at early growth stages, while genera *Burkholderia*, *Ralstonia*, *Dyella*, *Chitinophaga*, *Sphingobium*, *Bradyrhizobium* and *Variovorax* populations were dominant at later stages. Comparisons of pyrosequencing data collected in Illinois, USA in this study with the available data from Braunschweig, Germany indicated many common bacterial inhabitants but also many differences in the structure of bacterial communities, implying that some site-specific factors, such as soil properties, may play important roles in shaping the structure of rhizosphere bacterial community.

1. Introduction

The rhizosphere is directly influenced by root secretions and associated microorganisms. Soil pH, structure, oxygen and nutrition levels in the rhizosphere differ from those in the bulk soil (Singh et al., 2004). The unique ecological niche shapes the structure of rhizosphere bacterial community through the interactions of plant species, the chemical nature of root exudates, soil properties, and many other factors (Savka and Farrand, 1997). While some detrimental microbes undermine plant health, mutualistic rhizosphere microbes provide plants with mineral nutrients, phytohormones, and protect the plant against phytopathogens (Mendes et al., 2011; Singh et al., 2004; Vercellino and Gómez, 2013). Thus, to understand the suite of bacterial interactions that may occur over the lifetime of a plant, it is important to know the rhizosphere bacterial community and its variation over plant growth stages.

Rhizosphere bacterial communities differ across plant species, soil type, root architecture and growth stage (Berg and Smalla, 2009; Marschner et al., 2001, 2004). As maize (*Zea mays*) is an important crop, its rhizosphere bacterial community has been intensively investigated using a variety of approaches (Aira et al., 2010; Castellanos et al., 2009; Chelius and Triplett, 2001; Dohrmann et al., 2013). Previous studies suggested that maize selects for a specific bacterial community depending on soil properties (Castellanos et al., 2009), genotypes (Aira et al., 2010), crop management, such as fertilizer (Aira et al., 2010) and growth stages (Cavaglieri et al., 2009; Di Cello et al., 1997). Some studies have suggested that microbial community composition in the maize rhizosphere is independent of cultivar (Dohrmann et al., 2013; Schmalenberger and Tebbe, 2002), growth stage (Gomes et al.,

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2001) and genotype (Schmalenberger and Tebbe, 2002). The apparent disparity of bacterial responses in the rhizosphere could be caused partially by differences among plant species and soil types, but likely also by differences among the methodologies used, such as denaturing gradient gel electrophoresis (DGGE) and clone library analysis, which have different resolution and detection limitation (Bent and Forney, 2008). Low-resolution profiling methods potentially miss some important information and make it difficult to detail the phylogenetic composition of a rhizosphere bacterial community.

Plant growth stage will influence root physiology, and change the quality and quantity of root exudates; consequently, these changes exert a selection on root-associated microorganisms at different growth stages (Dunfield and Germida, 2003; Houlden et al., 2008). Some studies suggested that seasonal variations in the activity and relative abundance of rhizosphere bacterial communities are plant-dependent (Dunfield and Germida, 2003; Houlden et al., 2008; Mougel et al., 2006; Smalla et al., 2001). Although some research showed dynamic changes of maize rhizosphere bacterial community at different growth stages using culture-dependent (Cavaglieri et al., 2009; Nacamulli et al., 1997) and fingerprinting methods (Di Cello et al., 1997), detail phylogenetic compositions cannot be monitored due to low resolution of these approaches. It is also unclear at which taxonomic levels such dynamics can be detected.

In this study, we used DGGE and pyrosequencing methods to investigate the growth-stage related dynamics of the bacterial composition and diversity in the maize rhizosphere. The objectives are (i) to analyze the bacterial community structure and their growth-stage related dynamics in the rhizosphere of a maize cultivar, (ii) to compare the data of this study obtained from a fine-silty soil located in Illinois, USA, to those from a similar study conducted in a silty sand soil in Germany, in order to identify common bacterial inhabitants and the difference in the bacterial community structure.

2. Materials and methods

2.1. Field description and sampling

Our sample site was located at the Energy Farm of the University of Illinois (Champaign, IL, USA, 40°03′N, 88°12′W, 230 m elevation). The soil is a Drummer-Flanagan series (fine-silty, mixed, mesic Typic Endoaquoll, pH 5.8, organic matter 3.8%, total nitrogen 0.18%) formed from deep deposits of loess and silt parent material on the top of the glacial till and outwash plains. It is an organically rich, highly productive Corn Belt soil that has been rotation cropped with maize and legumes for over 100 years. The *Z. mays* cultivar *cv* 34B43 (Pioneer Hi-Bred International, Des Moines, IA, USA) was planted in the middle of May, 2011, in a 0.7-ha field and managed following standard agricultural practices in this region with annual applications of 20 g m^{-2} of mixed urea, ammonia, and nitrate fertilizer at planting.

Sampling was conducted after maize emergence at 2 weeks (June 1, 2011, vegetative stages V2–V3), 5 weeks (June 21, V8–V9), 9 weeks (July 18, VT, tasseling) and 12 weeks (August 11, R2, blister). Growth stage was defined based on Hanway's work (Hanway, 1963). We selected four plots in the maize field for sampling. Sampling sites for the next sampling were nearby the previous locations to minimize the effects caused by spatial variation of soil properties on the bacterial communities. Within each plot, 4 rhizosphere soil samples and 4 bulk soil samples were collected and composited, so that each individual sample was a pool of four plants or soil cores (1 m away for different plants or soil cores). In total, 16 bulk soil and 16 rhizosphere soil samples were obtained during the growing season (four plots sampled at four time points). To collect rhizosphere soil, plants were carefully excavated using a shovel, and visible soil particles were removed. Fresh roots were collected into a 50 ml centrifuge tube (ca. 50 g). Soil tightly attached on the root surface was rinsed using phosphate buffer solution, and centrifuged at 8000 rpm for 10 min. The pellet was collected as rhizosphere soil. Soil cores of 0–30 cm depth between plants were also collected as bulk soil samples. All the samples were transferred to the lab on dry ice, and pre-processed and stored at −80 °C for downstream applications.

2.2. DNA extraction

Soil genomic DNA was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA). DNA quality was assessed by the ratio of $A_{260/280}$ and $A_{260/230}$ using a nanoDrop spectrophotometer. DNA concentrations were also quantified with Qubit assay (Invitrogen, CA).

2.3. Denaturing gradient gel electrophoresis (DGGE)

PCR amplification of bacterial 16S rRNA genes from soil genomic DNA for DGGE analysis was done using the bacteria-specific forward primer 357F containing a GC-clamp and the reverse primer 907R (Muyzer et al., 1993). The PCR mixture (50 μl) contained 1× PCR buffer, 1.5 mM $MgCl_2$, each deoxynucleoside triphosphate at 0.4 μM, each primer at 1.0 μM and 1 U of *Ex Taq* polymerase (TaKaRa Bio Inc, Japan) and 20 ng soil genomic DNA. The PCR amplification program included initial denaturation at 94 °C for 3 min, followed by 30 cycles of 94 °C for 40 s, 56 °C for 60 s, and 72 °C for 60 s, and a final extension at 72 °C for 10 min. PCR products were subjected to electrophoresis using 1.5% agarose gel. The band with a correct size was excised and purified using Wizard® SV Gel and PCR Clean-Up System (Promega, USA).

DGGE was performed using a DCode system (Bio-Rad, California) as described by Muyzer et al. (1993). An 8% polyacrylamide gel with a linear denaturant concentration from 30 to 55% (where 100% denaturant contains 7 M urea and 40% formamide [v:v]) was used to separate the PCR products. Approximately 300 ng of PCR product per lane was loaded onto the DGGE gel. The gel was electrophoresed for 4 h at 60 °C at a constant voltage of 200 V, stained for 1 h with SYBR Green I (Molecular Probes, Eugene Org.), illuminated on a transilluminator under UV-light. The sharp bands were excised. 16S rRNA clone library was constructed using pGEM®-T Easy Vector Systems according to manufacturer's instruction (Promega, USA). Ten clones from each sample were sequenced using Sanger method.

DGGE images were analyzed with Quantity One software (Bio-Rad, USA). A matrix was constructed for all lanes, taking into account the presence or absence of the individual bands and the relative contribution of each band to the total intensity of the lane. A Dice coefficient similarity matrix was generated based on above matrix. Finally, DGGE profiles for all samples were clustered based on Dice's similarity coefficient using the complete-linkage method.

2.4. Pyrosequencing

To amplify the V4-V5 hypervariable regions of 16S rRNA genes for pyrosequencing, universal primers 519F (5′-CAGCMGCCGCGGTAATWC-3′) and 926R (5′-CCGTCAATTCMTTTRAGTT-3′) were used in PCR (Baker et al., 2003). The oligonucleotides included 454 Life Science's A or B sequencing adapter fused to the 5′ end of forward and reverse primers. A unique 10-mer barcode sequence was added between the sequencing adapter and forward primer to differentiate between samples. The methods for 16S rRNA gene amplification and