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Impact of transgenic Cry1Ac + CpTI cotton on diversity and dynamics of rhizosphere bacterial community of different root environments

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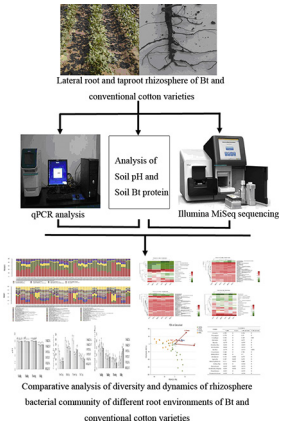
HIGHLIGHTS

- Total bacterial genera had similar pattern with dominant genera in abundance to the shifts in bacterial community structure.
- Total bacterial genera had similar pattern with rare genera in richness to the shifts in bacterial community structure.
- Dominant genera mainly contributed to dynamics of bacterial community composition.
- Soil pH was more correlated with variations in the bacterial community composition than Bt protein contents.
- No significant differences of bacterial community were detected in the same root zones between Bt and conventional cottons.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to characterize the diversity and dynamics of rhizosphere bacterial community, especially the response of dominant and rare bacterial taxa to the cultivation of Bt cotton for different root environments at different growth stages. qPCR analyses indicated that bacterial abundances of the taproots and lateral root rhizospheres of the Bt cotton SGK321 were significantly different at seedling and bolling stages. But no significant differences were detected between the same root zones from Bt and the conventional cotton varieties. Total bacterial genera had similar pattern with dominant genera in abundance, and with rare genera in richness to the changes of bacterial community, respectively. Although the rhizosphere bacterial diversity of the three cotton varieties changed in taproot and lateral root, no significant differences were detected in the same root environments between Bt and conventional cotton. Moreover, Soil pH was more correlated with variations in the bacterial community composition than Bt proteins. In conclusion, these results revealed no indication that rhizosphere bacterial community of Bt cotton had different response to increased Bt protein regarding the same root environment. In particular, dominant and rare bacterial taxa showed the variation in diversity and community composition in different root microhabitats.

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1. Introduction

In the mid-1990s, Bt cotton plant expressing larvicidal Cry proteins from *B. thuringiensis* was commercialized in the USA and China, and currently represents a major proportion of cotton plants in both the countries. To provide broader protection, Bt-cotton varieties with multiple insecticidal proteins have been developed. SGK321 is bred by introducing the synthetic *Cry1Ac* gene and modified *CpTI* (Cowpea Trypsin Inhibitor) gene into the cotton cultivar SY321 by way of the pollen tube pathway technique for protection against bollworms (Guo et al., 1999).

One of the major potential environmental risks associated with the use of Bt-cotton varieties is their effect on soil and its inhabiting non-target organisms, including bacteria (Dohrmann et al., 2013). Regarding the Cry protein released in root exudates from transgenic Bt plants throughout their growth (Saxena et al., 1999), it should be noted that Bt plants may modulate rhizosphere microbial diversity and possibly impact the functioning of the rhizosphere (Cotta et al., 2014; Lee et al., 2017). However, studies so far indicated that the rhizosphere microbial communities were hardly affected by the presence of Bt cotton plants (Miethling-Graff et al., 2010; Kapur et al., 2010; Li et al., 2011). Considering that these studies were based on a culture-dependent approach and genetic fingerprinting, the lack of detection may in fact be linked to the relatively low sensitivity of such methods. The natural microbial communities are typically composed of a few dominant species, along with a large number of rare taxa (Sogin et al., 2006). Rare taxa, in particular, have been hypothesized to play crucial roles in biogeochemical cycles and overall metabolic fluxes (Musat et al., 2008; Wrighton et al., 2012), because (i) their roles may be critical for the maintenance of soil functioning and (ii) they may be more responsive to environmental factors than high-redundancy groups (Cotta et al., 2014). Nowadays, high-throughput sequencing techniques such as Illumina MiSeq sequencing of short regions of the 16S rRNA gene allow examination of rare microbial populations (Sogin et al., 2006; Campbell et al., 2011). Accordingly, it is necessary to determine whether the changes of distribution dynamics of dominant and rare microbial taxa in the rhizosphere of GM cotton instead of non-GM cotton as a result of the modification in different growth stages varied.

The root system architecture (RSA) of cotton is typically composed of a primary (tap) root and lateral roots. It has been proposed that root branching and RSA play a significant role in determining the composition of exudates both quantitatively and qualitatively, resulting in a shift in the diversity of microbial communities (Badri and Vivanco, 2009). Although some studies show the effects of the consecutive cultivation of Bt cotton on soil microbe-mediated enzymatic properties and microbial biomass (Chen et al., 2017; Luo et al., 2017), no studies have been conducted to examine the effect of different root zones, along with Cry proteins released in the root exudates, of Bt cotton plants on the rhizosphere microbial community structure. Furthermore, there is exceptional importance to take into account the effect of root microhabitat of Bt plants, especially for dicotyledonous plants, on soil ecosystems.

The aim of this study was to investigate the diversity and dynamics of soil bacterial community to the cultivation of Bt cotton at different growth stages of the plant under field conditions, and how these are affected by additional Bt protein. The underlying hypotheses were: (1) bacterial community structure associated with different root environments of Bt cotton plant changed differently over time. (2) different growth stages of cotton plant affected the bacterial communities, especially the distribution of dominant and rare taxa. For this study, a total of 72 samples of Bt and conventional cotton varieties were collected at four growth stages, and their rhizosphere bacterial population sizes and community structures were studied using qPCR and 16S rRNA gene sequencing.

2. Materials and methods

2.1. Experiment design and soil sampling

GM cotton variety SGK321, which could produce insecticidal toxin Cry1Ac and Cowpea Trypsin Inhibitor (CpTI) protein, was generated by Shijiazhuang Academy of Agricultural and Forestry Sciences, China, from conventional cotton variety SY321. The control comprised conventional cotton varieties SY321 and XLZ13. All the cotton plants were cultivated on the field of Dezhou Academy of Agricultural Sciences, Shandong, China (37°21′ N, 116°20′ E). The fore crop of the experiment field was conventional wheat variety, and the wheat straw and litter were cleaned out before planting the cotton plants. The study site is located in the temperate monsoon climate zone with mean annual temperature of 15 °C and annual precipitation of 65 cm. The soil is classified as sandy loam soil, containing 11.23 ± 0.89 g/kg organic matter, 1.72 ± 0.08 g/kg of total nitrogen (N), 68.24 ± 5.78 mg/kg of alkhydr N, 17.54 ± 1.76 mg/kg of available P, 109.78 ± 8.78 mg/kg of available K, and pH of 7.22 ± 0.35 (soil/water ratio of 1:2.5). The study area comprised 30 m × 30 m per field, with three replicates of each treatment arranged in a randomized block experimental design. To avoid marginal effects, 10 m of land were spared at both the ends of each field. The experiment was conducted on March 30, 2016, according to the local conventional agriculture operations. Taproot and lateral root rhizosphere were sampled four times, corresponding to the major growth stages of cottons, namely seedling (July), budding (August), flowering (September), and bolling (October) stages. After weeds and leaves were removed from the soil surface, plants were gently pulled out and the rhizosphere soil was collected by gently shaking the roots to dislodge small adhering soil clumps. To ensure representativeness of the samples, each sample was collected from five different sampling points within a distance of 5 m (Li et al., 2014) and in triplicates on each sampling day. A part of the soil sample was stored at −80 °C and used for DNA extraction, while the remaining part was used for the analysis of soil properties and Bt protein content measurements.

2.2. Quantification of Cry-proteins from rhizosphere

Add up to 0.5 g of soil sample to a 2-mL microcentrifuge tube. Next, 1.0 mL extraction buffer (0.1 M Na_2CO_3, 0.1 M $NaHCO_3$, 5.0 mM EDTA, 50 mM $Na_4P_2O_7 \cdot 10H_2O$, 0.1%Triton X-100, pH 10) was added to fill the tube and the sample was mixed for 30 min at room temperature, followed by centrifugation for 5 min at 4 °C at 12,000*g*. The supernatant was collected to measure Bt protein content using a commercial ELISA kit (QuantiPlate™ Kit for Cry1Ab/Cry1Ac, EnviroLogix Inc., Portland, Maine, USA) according to the manufacturer's protocol.

2.3. Analysis of soil pH and soil microbial DNA extraction

The soil pH was determined by pH meter (FE20-FiveEasy™ pH, Mettler Toledo, German) at a ratio of 1: 2.5 (weight/volume) for soil versus distilled water. Microbial DNA of the soil samples was extracted using the FastDNA spin kit for soil (MP Biomedicals, LLC, Solon, USA). Purified DNA was stored at −80 °C for the analyses of qPCR and Illumina MiSeq sequencing.

2.4. Quantification of the bacterial community

The population sizes of the rhizosphere bacterial communities were determined by qPCR using 10 μl of reaction mixture comprising 5 μl of 2× SYBR qPCR Premix Ex Taq™ (TaKaRa Biotechnology (Dalian) Co., Ltd) and 0.25 μl each of 10 mM universal bacterial 16S rRNA gene primers 1369F and 1492R (Suzuki et al., 2000). A total of 4 μl of template DNA diluted 10-folds in TE buffer (10 mM Tris and 1 mM EDTA, pH 8) and 0.5 μl of deionized distilled water were used in 10 μl reaction volume. All the samples were analyzed in triplicates, and the amplification