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Trichoderma harzianum T-E5 significantly affects cucumber root exudates and fungal community in the cucumber rhizosphere

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ABSTRACT

The Fusarium wilt in cucumbers, caused by the pathogenic fungus *Fusarium oxysporum* f. sp. *cucumerinum*, is a serious and destructive disease worldwide. An effort was made to explore the role of *Trichoderma harzianum* T-E5 in reducing the incidence of Fusarium wilt. Three treatments (Control, T1, and T2) were established in the greenhouse experiment. The effects of T-E5 on the composition of root exudates and fungal community in the cucumber rhizosphere were measured. Compared with the control, the application of a bio-organic fertilizer (BIO) enriched with T-E5 was found to decrease the incidence of Fusarium wilt notably and promote the growth of cucumber plants. Based on real-time PCR, the population of *F. oxysporum* in the control without T-E5 increased from 10^3 to 10^4 ITS copies g^{-1} soil, whereas the population decreased from 10^3 to 10^2 ITS copies g^{-1} soil in the T1 and T2 treatment groups when T-E5 was included. Significant difference in fungal community was also found among the treatment groups. HPLC analysis showed that the detected levels of phenolic compounds in control were significantly higher than the levels in the samples subjected to T1 and T2 treatments. The root exudates from the control group significantly increased the numbers of germinating spores of the pathogen compared with those from the samples treated with T1 and T2. In conclusion, the modification of root exudates and the fungal community by the application of BIO might account for the effective suppression of Fusarium wilt disease in cucumbers.

1. Introduction

Soil microbial diversity and community structure have a great influence of plants to resist disease (Garbeva et al., 2004; Van Bruggen et al., 2006). However, poor farming practices, such as continuous cropping can cause the alteration of the soil microbial community and increase the level of phytopathogenic fungi, such as *Fusarium* spp. (Chen et al., 2012) and *Rhizoctonia solani* (Huang et al., 2011). The soil-borne pathogen *Fusarium oxysporum* f. sp. *cucumerinum* (Cao et al., 2012; Chen et al., 2010; Li et al., 2012), which mainly causes Fusarium wilt in cucumbers, is responsible for restricting cucumber production and quality worldwide (Ye et al., 2004). Crop rotation or the application of organic fertilizers can change the soil microbial diversity and community structure (Yin et al., 2010; Huang et al., 2011). Many microbes have been reported to be potential bio-control agents in suppressing soil-borne plant diseases (Abawi and Widmer, 2000; Van Elsas et al., 2002). Therefore, combination of organic fertilizers and bio-control agents is a good way to control plant disease.

The effective biocontrol of Fusarium wilt has been achieved by using fungi belonging to the genera *Trichoderma* spp., which are the most prevalent and culturable fungi in the soil. These fungi have also been widely reported to degrade the allelochemicals of continuously cropped cucumbers (Chen et al., 2011a, 2011b), and they show a potent antagonistic effect against a variety of soil pathogens (Howell et al., 2000; Papavizas, 1985). Until now, little information has been available on the modification of root exudates by inoculating plants with *Trichoderma* spp. or by their combination with organic fertilizers.

The root exudates are released from the plant tissues during the growth age (Asao et al., 2003; Chou, 2010) and have a significant influence on the soil properties, microbial communities and soil functions (Hopkins et al., 1998; Shi et al., 2011). The kinds of root exudates are dependent on many factors such as crop species, growth ages, elemental composition of the soil and microorganism effects (Steinkellner et al., 2005). The composition of the root exudates mainly include water, ions, enzymes, sugars, phenolic compounds, amino acids and organic acids (Bertin et al., 2003).

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Phenolic compounds such as ferulic acid, benzoic acid, cinnamic acid, adipic acid, vanillic acid and *p*-hydroxybenzoic acid have been identified from cucumber and taro plants (Asao et al., 2003; Hu et al., 2007; Chen et al., 2011a, 2011b). These compounds are involved in many plant metabolic processes, including photosynthesis, respiration (Weir et al., 2004), the regulation of membrane permeability (Baziramakenga et al., 1995), the acquisition of nutrients (Balke, 1985) and the modification of soil properties (Bertin et al., 2003). Plants may produce autotoxic phenolic compounds in response to an attack by pathogens (Ma et al., 2005). Ye et al. (2004) demonstrated that the cucumber released benzoic acid and cinnamic acid, which aggravate the Fusarium wilt. Similar phenomena are also observed in the tomato (Steinkellner et al., 2005). The concentration of a phenolic compound determines whether a pathogen proliferates (Yuan et al., 2004). Generally, low concentrations of a phenolic compound enhance the growth of a microorganism, whereas high concentrations of it inhibit the growth (Ling et al., 2011). However, Wu et al. (2009) confirmed that benzoic acid at any concentration can strongly inhibit the growth, sporulation and conidia germination of *F. oxysporum* f. sp. *niveum*.

We hypothesized that the root exudates would change and had an inhibitory effect on the conidia germination of *F. oxysporum* f. sp. *cucumerinum* when the cropped soil was treated with *Trichoderma harzianum* T-E5 bio-organic fertilizer (BIO), with a more significant effect as the application rate was increased. Correspondingly, both the composition of root exudates and microbial diversity would be modified in the cropped soil applied with BIO. Therefore, the objective of the present study was to evaluate the effects of *T. harzianum* T-E5 on the composition of root exudates and rhizosphere fungal community for obtaining the relationships among T-E5, root exudates and soil microbial community, and expecting to provide evidence for Fusarium wilt in cucumbers and to find a more efficient way to overcome Fusarium wilt.

2. Materials and methods

2.1. Fungal strains and culture conditions

The pathogen used throughout this study was *F. oxysporum* f. sp. *cucumerinum* (Agricultural Culture Collection of China (ACCC) No. 30220), which was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The antagonistic strain was *T. harzianum* T-E5 (Zhang et al., 2012), which is a putative mutant. Both T-E5 and *F. oxysporum* were maintained in 30% glycerol at −80 °C and grown on a potato dextrose agar (PDA) medium at 28 °C.

2.2. Preparation of fungal microconidia

A T-E5 conidia suspension was prepared by soaking PDA medium plates for 7 d at 28 °C in the dark to induce sporulation. The plates were drenched with sterile water and carefully scraped with a sterile glass hockey stick. The suspension was filtered through four layers of sterile cheese cloth, and then the conidia concentration was determined based on hemacytometer counts, in which the final concentration was 2.4×10^7 cfu mL^{-1}. The conidia suspension of *F. oxysporum* was similarly prepared and the concentration of *F. oxysporum* was adjusted to 3.2×10^3 cfu mL^{-1}.

2.3. Preparation of bio-organic fertilizers (BIOs)

Organic fertilizer (OF), which is a mixture of an amino acid fertilizer and pig manure compost in a 1:1 weight ratio, used in this study was similar to the fertilizer used by Lang et al. (2012), and the BIO was made as described by Ling et al. (2010). Briefly, BIO was created by placing 100 mL T-E5 conidia suspension (2.4×10^7 cfu mL^{-1}), 500 mL sterile water and 5 kg OF together. And they were mixed in a large sterile container and stirred thoroughly to sustain the growth of T-E5, maintaining the secondary solid fermentation (for 7 d) at 30–40 °C and moisture at 40–45%. Then, the BIO was air dried, and the final dry weight density of BIO was 3.2×10^6 cfu g^{-1}.

2.4. Greenhouse experiments

2.4.1. Greenhouse experiment design

The experiment had a completely randomized block design with three replications that had the following treatments: T1 (soil inoculated with 0.5% BIO) and T2 (soil inoculated with 1% BIO), while a control group was only amended with 0.5% OF. The replication in each treatment group consisted of 15 total pots. Every three cucumber seedlings in each replication at different time points (10, 20, and 30 d following transplantation) were sampled as one sample for rhizosphere soil total genomic DNA extraction and root exudates collection. And at 30 d, all the rest cucumber seedlings were harvested. The disease incidence, plant dry weight and shoot length were also evaluated. The disease incidence was shown as the percentage of infected cucumber seedlings over the total number in each replication (Cao et al., 2011).

2.4.2. Soils used in the experiment

The nursery soil for the growth of cucumber seedlings was collected from Yixing, Jiangsu, China and had not been planted with cucumbers previously, while the potting soil was collected from Weihui, Henan, China and had been used to grow cucumber for four successive years. The nursery soil contained 13.7 g kg^{-1} organic matter, 1.62 g kg^{-1} total N, 18.9 mg kg^{-1} available P, 128 mg kg^{-1} available K, acidity at pH of 6.8, and none of *F. oxysporum* in culture plate. In contrast, the potting soil had encountered Fusarium wilt with a more than 80% of disease incidence in field due to continuous cropping in 2011. The potting soil contained 11.8 g kg^{-1} organic matter content, 1.02 g kg^{-1} total N, 20.1 mg kg^{-1} available P, 47 mg kg^{-1} available K, acidity at pH of 6.6, and spores of *F. oxysporum* at a level of 2.02×10^3 cfu g^{-1} dry soil.

2.4.3. Cucumber seedlings and growth conditions

The cucumber seeds (*Cucumis sativus* L.), a cultivar of JinChun-No.4 obtained from the Tianjin Cucumber Research Center, were used in this experiment. The seeds were washed with sodium hypochlorite (2%, v/v) for 5 min and rinsed several times with sterilized distilled water. All seeds were germinated in the dark at 28 °C for 36 h. After germination, the seeds were sowed into nursery cups and each cup was filled with 300 g sterilized nursery soil and maintained for 10 d. Next, the cucumber seedlings were removed into pots with 3 kg potting soil, in which a single seedling was planted in each pot. The entire experiment was conducted in a greenhouse, located in Yixing, China, from July to August 2011, with a temperature of 28–35 °C and a relative humidity of 60–70%.

2.5. Extraction of rhizosphere soil total genomic DNA

The cucumber seedlings were sampled 10, 20 and 30 d after transplantation as described in Section 2.4.1. The cucumber seedlings were taken out of the pots carefully and shaken lightly to remove most of the soil. The soil still adhering to the roots (rhizosphere soil) was collected and sieved carefully (Smalla et al., 2001). The total genomic DNA of the rhizosphere soil samples were extracted using the EZNA^{TM} Soil DNA kit (Omega Bio-Tek, Inc., CO, USA) and stored at −20 °C.