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Responses of cucumber rhizosphere communities of *Fusarium* and *Bacillus* spp. to exogenously applied *p*-Coumaric acid

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ABSTRACT

In monocropping systems autotoxins, such as phenolic compounds, could accumulate in soil and affects the soil microbial communities, however, the specific effects on microbial species are unknown. We determined the responses of cucumber rhizosphere *Fusarium* and *Bacillus* spp. communities to exogenously applied *p*-coumaric acid (0.02, 0.05, 0.1, 0.2 μmol/g soil) by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) technique. Results showed that all concentrations of *p*-coumaric acid changed the community structure of *Fusarium* spp. and *p*-coumaric acid at 0.2 μmol/g soil decreased the number of bands as indicated by Shannon-Wiener and evenness index of *Fusarium* spp. community. However, *p*-coumaric acid did not affect the structure of *Bacillus* spp. community. These results indicated that the *p*-coumaric acid showed different effects on *Fusarium* and *Bacillus* spp. communities.

Keywords: Autotoxins, *Bacillus* spp., community structure, cucumber, *Cucumis sativus, Fusarium* spp., monocropping, *p*-coumaric acid, PCR-DGGE, rhizosphere

INTRODUCTION

Plants secretes a diverse range of compounds as root exudates in rhizosphere during subtle belowground interactions (1,2,23). These phenolic compounds are important secondary metabolites, which play multifunctional roles in plant-plant and plant-microbe interactions (4). In monocropping, when the same crop is repeatedly grown on the same piece of land, it results in accumulation of autotoxins such as phenolic compounds in the soil (21). Earlier studies have shown that phenolic compounds of corn (*Zea mays L.*) (10), tea (*Camellia sinensis*) (11) and cucumber (*Cucumis sativus L.*) (31) have autotoxic effects.

Soil microorganisms are an important factor to determine soil health and the main driving force of soil sickness (13). The plant health largely depends on activities and functions of rhizosphere microbiome, which are quite essential for the sustainability of agroecosystems (25,27,29). *Bacillus* and *Fusarium* spp. are ubiquitous in terrestrial ecosystem and they are abundantly present in association with plants, either as saprophytes, mutualists or pathogens (9,15). Studies have shown that most of the *Bacillus* spp. improves plant health by facilitating nutrients mobilization and inducing systematic resistance (17). In addition, *Bacillus* spp. improves the chickpea plant growth by directly inhibiting the *F. oxysporum f. ciceris*, a soil born pathogen causing vascular wilt disease in chickpea (16). However, *Fusarium* spp. is mostly plant pathogenic and causes huge losses in vegetables production (12,14). Studies have shown that certain land management practices such as

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[#]Co-first authors, *Correspondence author intercropping or crop rotation could alter the soil *Bacillus* and *Fusarium* spp. communities (29,30), but little is known about the effects of specific phenolic compounds on *Bacillus* and *Fusarium* spp. in soil.

Previous studies have confirmed that *p*-coumaric acid inhibited the cucumber growth and changed the rhizosphere soil microbial communities. However, the effects of *p*-coumaric acid on the diversity and community structure of *Bacillus* and *Fusarium* spp. remains unclear. Hence this study, aimed to determine the effects of *p*-coumaric acid on *Bacillus* and *Fusarium* spp. communities in a pot culture experiment by using polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) technique.

MATERIALS AND METHODS

Greenhouse experiment

The soil for this experiment was collected from the undisturbed upper layer (0-15 cm) of an open field of our Horticulture Experimental Station, Northeast Agricultural University, Harbin, China (45°41'N, 126°37'E, mean height above sea level: 127.95 m, annual precipitation: 524.5 mm, minimum -maximum temperature: -37.7°C, 36.7°C). The soil was sandy loam having organic matter: 3.67 %, available N: 89.02 mg/kg, available P: 63.36 mg/kg, available K: 119.15 mg/kg, EC (1:2.5, w/v): 0.33 mS/cm, and pH (1:2.5, w/v): 7.78.

Cucumber (cv. Jinlv 3) seedlings with two cotyledons, were transplanted into pots containing 150 g soil. As the phenolic compounds added to the soil could be quickly degraded (21). Therefore, *p*-coumaric acid was added to the soil periodically to maintain the desired levels as described before (26). *p*-Coumaric acid was purchased from Solarbio Life Science Company, Beiing, China. Cucumber seedlings at one-leaf stage, (10 days after transplanting), were treated 5-times with *p*-coumaric acid at 0, 0.02, 0.05, 0.1, 0.2 μmol/g soil concentrations every 2-days. Each treatment had 5-pots and replicated thrice. As the pH is an important factor that affects soil microorganisms (18), the pH of phenolic compounds solution was adjusted to 7.0 with 0.1 mol /L NaOH solution. The cucumber seedlings were placed in the greenhouse and watered every 2-days to maintain soil moisture level about 50±5 % of water holding capacity. After 10 days, the soil was collected from each treatment, passed through a 2 mm sieve and stored at -80°C for DNA extraction.

DNA extraction and PCR-DGGE

Total soil DNA was extracted using the Power Soil® DNA Isolation Kit (MO BIO Laboratories, CA, USA) according to the manufacturer's protocol. The community structures of *Fusarium* and *Bacillus* spp. in soils were determined by PCR-DGGE with the primer set EF-1/EF-2 (20) and Alfie1/Alfie2 (24) for *Fusarium* spp. (2), and BacF/BacR and GC-338f/518r for *Bacillus* spp. (9). Denaturing gradient gel electrophoresis (DGGE) was performed by using an 8 % (w/v) acrylamide gel with 20-60 % denaturant gradient for *Bacillus* spp. and 6 % (w/v) acrylamide gel with 40-60 % denaturant gradient for *Fusarium* spp. Electrophoresis was done with a DCode universal mutation detection system (Bio-Rad Lab, LA, USA) at 60 °C and 80 V for 14 h in 1 x TAE (Tris-acetate-EDTA) buffer. After electrophoresis, the gel was stained in a 1: 3300 (v/v) GelRed (Biotium, CA, USA) nucleic acid staining solution for 20 min. The DGGE profiles were photographed under UV light using an AlphaImager HP imaging system (Alpha Innotech Crop., CA, USA)

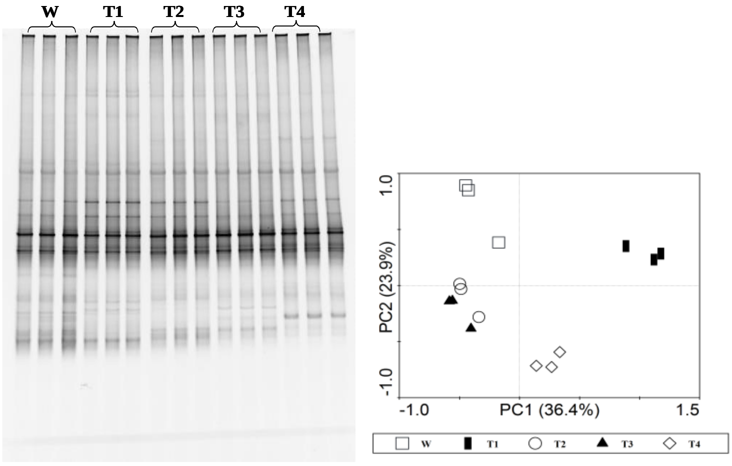
Data Analysis

Data were analyzed by ANOVA using SAS 8.1 software and an average comparison between the treatments was done based on Tukey's Honestly Significant Difference (HSD) test at a 0.05 probability level. DGGE bands were analyzed with Bio-Rad Quantity One software (version 4.5). Principal component analysis (PCA), calculation of DGGE band number, Shannon-Wiener index (H) and evenness index (E) (19) were calculated using Canoco for Windows 4.5 software as previously described (22).

RESULTS AND DISCUSSION

Fusarium spp. community structure

Treatment with *p*-coumaric acid at of 0.02-0.2 µmol concentration significantly reduced the number of bands in the DGGE map of the Fusarium community, the Shannon-Wiener index (H) and evenness index (E) treated with 0.2 µmol *p*-coumaric acid were lower than other treatments (Figure 1). Therefore, various concentrations of *p*-coumaric acid had different effects on the community structure of Fusarium in the soil. *Fusarium* spp. is a filamentous ascomycete fungus of importance to agriculture, environment and human health (28). A number of *Fusarium* spp. are phytopathogenic that infects many crops and cause vascular wilt disease (17). The *in-vitro* inhibitory effects of p-coumaric acid on *F. oxysporum* f.sp. *niveum* have been shown (11), which were consistent with our results. Thus, we speculated that addition of *p*-coumaric acid might reduce the occurrence of cucumber diseases.



(a) (b)

Figure 1. DGGE spectrum of *Fusarium* spp. community treated with *p*-coumaric acid (a) and its principal component analysis (b).
Note: W represents the control treatment; T1, T2, T3, and T4 represent the *p*-coumaric acid treatment of 0.02, 0.05, 0.1, and 0.2 µmol/g soil, respectively.

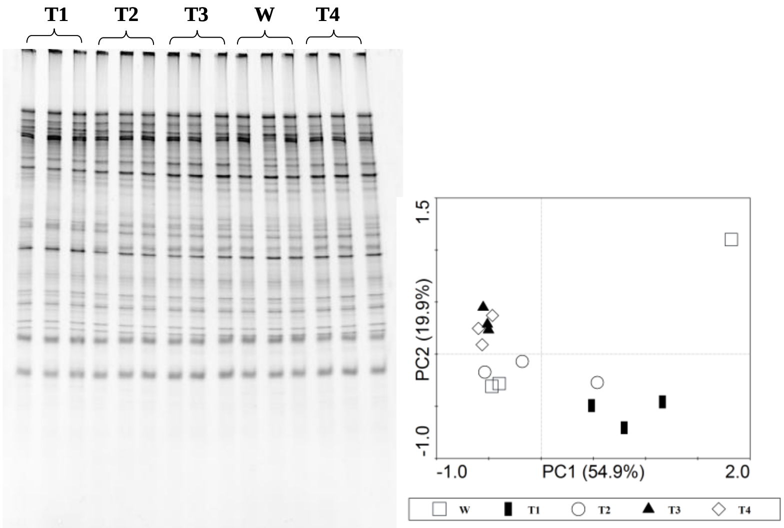
***Bacillus* spp. Community structure**

The treatments of *p*-coumaric acid at all concentrations (0.02, 0.05, 0.1, 0.2 μmol/g soil) did not affect the number of DGGE bands, Shannon-Wiener and evenness index values of *Bacillus* spp. ($P < 0.05$) (Table 1). PCA analysis showed that the PC1 and PC2 components together accounted for 65.5 % of the variation. However, the five treatments were not clearly different from each other on the PCA plot (Figure 1b).

Table 1. Effects of *p*-coumaric acid on the number of DGGE bands (S), Shannon-Wiener index (H) and evenness index (E) of *Bacillus* and *Fusarium* spp. community.

p-coumaric acid Conc*	*Bacillus* spp. community			*Fusarium* spp. community		
	S	*H*	*E*	*S*	*H*	*E*
0	17.00±1.73 a	2.70±0.13 a	0.90±0.04 a	14.67±1.15 a	2.53±0.13 a	0.87±0.05 a
0.02	16.33±0.58 a	2.68±0.02 a	0.89±0.01 a	12.00±0.00 b	2.34±0.04 ab	0.81±0.01 ab
0.05	16.33±0.58 a	2.69±0.04 a	0.90±0.01 a	11.33±0.58 b	2.33±0.06 ab	0.81±0.02 ab
0.1	16.00±1.00 a	2.64±0.05 a	0.88±0.02 a	11.67±0.58 b	2.37±0.06 ab	0.82±0.02 ab
0.2	16.33±0.58 a	2.67±0.03 a	0.89±0.01 a	10.33±0.58 b	2.15±0.09 b	0.74±0.03 b

Note: The *p*-coumaric acid test concentrations were 0.02, 0.05, 0.1, and 0.2 μmol/g soil.
S: DGGE bands, H: Shannon-Wiener index, E: Evenness index



(a) (b)

Figure 2. DGGE profile (a) and PCA (b) analysis of *Bacillus* spp. community
Note: W represents control; T1, T2, T3, and T4 represent *p*-coumaric acid at the concentration of 0.02, 0.05, 0.1, and 0.2 μmol/g soil, respectively.

In this experiment, *p*-coumaric acid changed the community structure of *Fusarium* spp. significantly at 0.2 μmol concentration, but did not change the community structure of *Bacillus* spp. (Fig. 2). It has been shown that *p*-coumaric acid did not significantly affect the community structure of *Trichoderma* spp. (8), this indicated that phenolic acids in soil selectively affects the types of certain microbial communities in the rhizosphere. Simultaneously, we believed that under the influence of coumaric acid, *Bacillus* spp. might produce dormant spores due to the poor environment, which needed to be discussed in subsequent experiments.

CONCLUSIONS

p-Coumaric acid affected these two microbial communities *Fusarium* and *Bacillus* spp. differently as it changed the community structure and decreased the α-diversity of *Fusarium* spp. but not of *Bacillus* spp. However, response of certain species against *p*-coumaric acid and the effects of other phenolic compounds on *Bacillus* spp. community need to be studied in future.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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PUBLISHER NOTE