

Effects of selected cucumber root exudates components on soil *Trichoderma* spp. communities

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ABSTRACT

In a microcosm experiment, soils were treated with selected cucumber root exudates components (glucose, succinic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid and glutamic acid) at 20 µg C/g soil. The Soil *Trichoderma* spp. community structure and abundance were estimated by PCR-denaturing gradient gel electrophoresis and quantitative PCR, respectively. Results showed that all exogenously added organic compounds changed the structure and increased the diversity indices of soil *Trichoderma* spp. community. Among all tested organic compounds, glucose had the strongest, while, *p*-hydroxybenzoic acid had the weakest influence on soil *Trichoderma* spp. community structure. *p*-Hydroxy benzoic acid, glucose, succinic acid and glutamic acid increased the soil *Trichoderma* spp. community abundance with glucose being the best.

Key words: Amino acid, microcosm experiment, organic acid, sugar, *Trichoderma* spp.

INTRODUCTION

In terrestrial ecosystems, higher plants release significant amounts of photosynthetically fixed carbon into the rhizosphere through root exudates (2,12). These include low molecular weight compounds (organic acids, amino acids and sugars) and high molecular weight compounds such as mucilage and proteins (2,22). Plants use root exudates as chemical signals to monitor and interact with their surroundings and root exudates play important roles in plant-plant and plant-microbe interactions (2,23,24,30,39). For example, one plant species can inhibit the growth of another species through its root exudates, a phenomenon known as allelopathy (16). There are numerous microorganisms in the soil and plant-microbial interactions can strongly influence the plants performance (21,29,34,46,47). Easily available organic compounds in root exudates stimulate the microorganisms (2,37). Moreover, through root exudation, plants can select microorganisms in the rhizosphere to their own benefit, deter pathogenic microorganisms, while, attracting the mutualistic organisms (6).

Fungi show the greatest eukaryotic diversity on the planet and they are one of the primary decomposers in the ecosystem (1,3,5). Fungi can behave as mutualists as well as pathogens of plants and have great influences on the crop productivity in agroecosystems

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(3,10,28,46). *Trichoderma* spp. are opportunistic, avirulent plant symbionts, as well as are fungal parasites and have the ability to control the plant pathogenic fungi and promote the plant growth and development (15,25,26). Previous studies showed that *Trichoderma* spp. can metabolize organic acids, such as oxalic, malic and citric acids (41) and plant-released sucrose plays an important role in symbiotic association between *Trichoderma virens* and maize (36). However, the influences of different plant root exudate components on *Trichoderma* spp. in soil is still not clear.

Cucumber (*Cucumis sativus* L.) is a popular vegetable that is continuously cropped under greenhouse conditions (32,50). Root exudates from cucumber contains sugars such as glucose and fructose, organic acids such as citric, malic and succinic acids and phenolic acids, amino acids serine, glutamic acid aspartic acid and phenolic acids (20,30,35,40). Phenolic acids, such as *p*-hydroxybenzoic and *p*-coumaric acids are phytotoxic to cucumber growth (30,40). Previously, we found that phenolic acids were able to change the cucumber rhizosphere soil microbial and *Trichoderma* spp. communities (17,38,42,49,52). In this study, we further evaluated the effects of selected main components of cucumber root exudates on the soil *Trichoderma* spp. communities.

MATERIALS AND METHODS

Microcosm experiment

The soil used was collected from the undisturbed upper soil layer (0-15 cm) of an open field in our 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, maximum and minimum temperature: 36.7°C, -37.7°C). The soil was sandy loam, contained 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 (43).

Microcosm experiment was performed in flasks containing 60 g fresh soil (sieved through 2-mm mesh). To stabilize the soil microbial communities, these soils were pre-incubated at 20°C in the dark for five days with soil water content maintained at about 50% of its water holding capacity. Then, ammonium nitrate (NH₄NO₃) solution was added to the soils at 450 µg N/g soil to avoid potential microbial growth limitation by nitrogen (31). Organic compounds were added into the soil periodically as described before (31). Glucose, succinic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid and glutamic acid were added to the soil 20 µg C/g soil every two days for five times. Soils treated with distilled water served as control. All added solutions were uniformly mixed with the soil after each addition to avoid concentration gradients. Each treatment had five flasks and replicated thrice. The solution pH was adjusted to 7.0 with 0.1 M NaOH because the soil pH is a dominant factor that regulates soil microbial communities (13). Flasks containing these treated soils were sealed with Parafilm (Bemis Company, Inc., Wisconsin, USA) and incubated at 20°C in the dark. Soil water content was maintained at about 50% of its water holding capacity. All organic compounds used in this study were purchased from Solarbio Life Science Company, Beijing, China.

Soil sampling and DNA extraction

One day after the fifth application (11th day) of organic compounds, ten g fresh soils were sampled from each flask with a sterilized lab spoon and soils from five flasks in each replicate were mixed to make a composite sample. Total soil DNA was extracted with the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, USA) as per the manufacturer's instructions.

PCR-DGGE analysis

Trichoderma spp. community structure was analyzed by PCR-DGGE method. *Trichoderma* spp. partial ITS region was amplified with nest PCR protocols as described before (45). Primer sets of ITS1F/ITS4TrR and ITSTrF-GC/ITSTrR were used in the first and second round of PCR amplifications, respectively (27). DGGE analysis was performed on a 6% (w/v) acrylamide gel with 40-60% denaturant gradient. The gel was electrophoresed, stained and photographed as described before (45).

Quantitative PCR assay

Trichoderma spp. community abundance was estimated by SYBR Green qPCR assays with an IQ5 real-time PCR system (Bio-Rad Lab, LA, USA) as described before (45,52). *Trichoderma* spp. partial ITS regions were quantified with primer set of uTr/uTr (14). Standard curves were created with 10-fold dilution series of plasmids containing the ITS regions from soil samples. Threshold cycle (Ct) values obtained for each sample were compared with the standard curve to determine the initial copy number of the target gene. Sterile water was used as a negative control to replace the template. All amplifications were performed in triplicate.

Statistical analysis

Banding patterns of the DGGE profiles were analyzed using Quantity One V4.5 as described before (50). Principal component analysis (PCA) was used to compare the banding patterns between samples with normalized data using Canoco for Windows 4.5 software (44). Analysis of similarities (ANOSIM) was used to test for the overall effect of treatment on microbial community structures using the Vegan package in 'R'. Bray-Curtis distances among treatments were calculated using the Vegan package in 'R'. The microbial community diversity indices, including number of bands, Shannon-Wiener index and evenness index, were calculated as described before (44). Data were analyzed following analysis of variance (ANOVA) and mean comparison between treatments was performed based on the Tukey's honestly significant difference (HSD) test at 0.05 probability level.

RESULTS AND DISCUSSION

***Trichoderma* spp. community structure**

DGGE banding patterns of the *Trichoderma* spp. community were similar in triplicate samples of each treatment (Fig 1a). However, there were differences among treatments, both in terms of presence/absence of individual DGGE bands and the intensity of co-migrating DGGE bands.

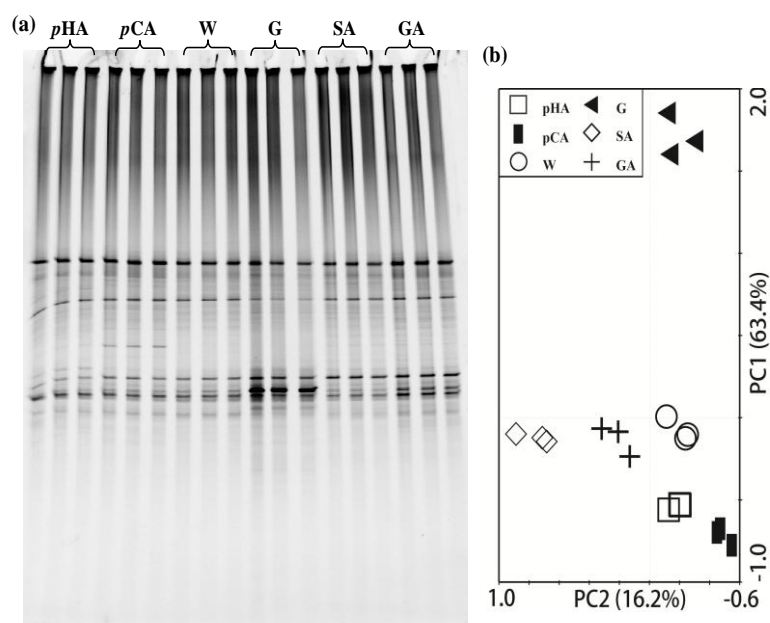


Figure 1. PCR-DGGE profile (a) and PCA analysis (b) of soil *Trichoderma* spp. community. pHA, pCA, G, SA, GA and W represent soils treated with *p*-hydroxybenzoic acid, *p*-coumaric acid, glucose, succinic acid, glutamic acid and water, respectively.

On the PCA plot of soil *Trichoderma* spp. community DGGE profiles, the first two axes together accounted for 79.6% of the total variation (Fig. 1b). PCA analysis clearly separated all the treatments from each other (Fig. 1b). ANOSIM analysis also confirmed that *Trichoderma* spp. community structure significantly differed among the treatments ($R=0.999$, $P=0.001$). On the PCA plot, among all treatments of organic compounds, the treatment with glucose had the furthest distance with the treatment of water (Fig. 1b). The Bray-Curtis distance between the treatment of water and glucose was larger than those between the treatments of water and other organic compounds ($P < 0.05$) (Fig. 2a). Moreover, the Bray-Curtis distance between the treatment of water and *p*-hydroxybenzoic acid was smaller than between the treatment of water and treatments of *p*-coumaric acid, succinic acid and glutamic acid ($P < 0.05$). Therefore, glucose had the strongest while *p*-hydroxybenzoic acid had the weakest influence on *Trichoderma* spp. community structure among all tested organic compounds. These results were in contradictory to the observation that succinic and glutamic acids had stronger influence on the whole bacterial community structure (unpublished data).

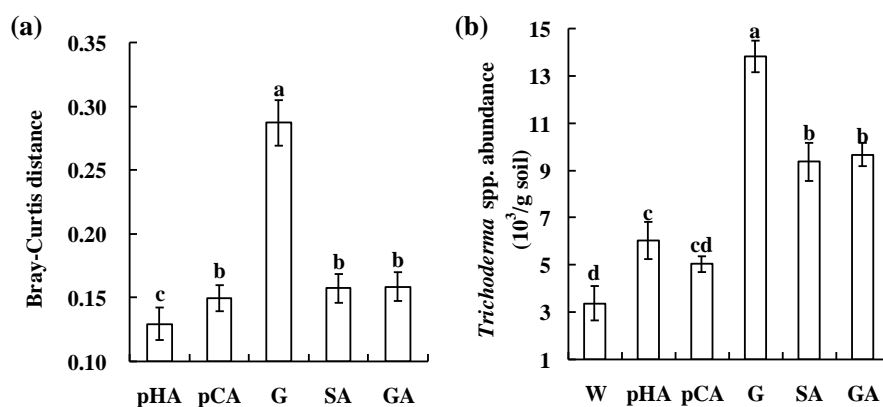


Figure 2. Bray-Curtis distances between control and treatments of organic compounds (a) and abundance (b) of soil *Trichoderma* spp. community.

pHA, pCA, G, SA, GA and W represent soils treated with *p*-hydroxybenzoic acid, *p*-coumaric acid, glucose, succinic acid, glutamic acid and water, respectively. Values with different letters were significantly different between treatments ($P < 0.05$, Tukey's HSD test).

All organic compounds increased the number of bands, Shannon-Wiener and Evenness indices (50) of soil *Trichoderma* spp. community ($P < 0.05$) (Fig. 3). The *p*-hydroxybenzoic acid treatment had lower number of bands, Shannon-Wiener and Evenness indices than treatments with *p*-coumaric acid, glucose, succinic acid and glutamic acid ($P < 0.05$). Moreover, succinic acid treatment had higher Shannon-Wiener and Evenness indices than treatments of *p*-coumaric acid and glucose ($P < 0.05$).

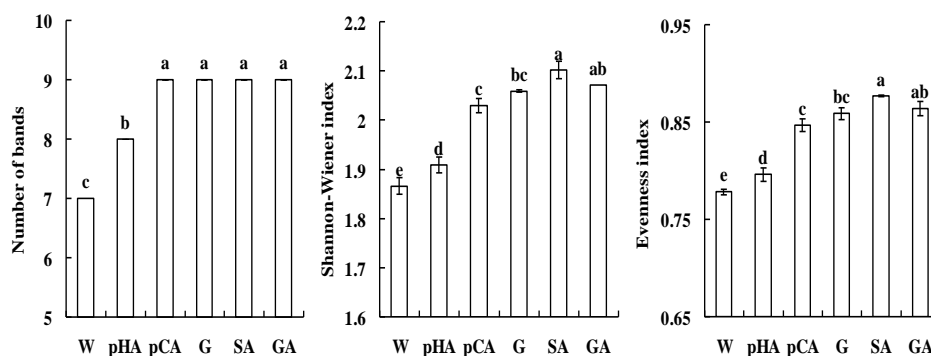


Figure 3. Effects of selected root exudate components on soil *Trichoderma* spp. community diversity. pHA, pCA, G, SA, GA and W represent soils treated with *p*-hydroxybenzoic acid, *p*-coumaric acid, glucose, succinic acid, glutamic acid and water, respectively. Values with different letters were significantly different between treatments ($P < 0.05$, Tukey's HSD test).

***Trichoderma* spp. community abundance**

All organic compounds, except *p*-coumaric acid, increased the soil *Trichoderma* spp. community abundance ($P < 0.05$) (Fig 2b). Among all treatments, the treatment with glucose had the highest abundance of soil *Trichoderma* spp. community abundance ($P < 0.05$). Succinic acid and glutamic acid treatments had higher soil *Trichoderma* spp. community abundance than the *p*-hydroxybenzoic acid and *p*-coumaric acid treatments ($P < 0.05$). However, the *in-vitro* effects of these compounds on *Trichoderma* spp. are not clear, which should be investigated in the future.

Root exudates can serve as selective agents, through which a plant regulates the microbial community in its surrounding rhizosphere (2,52). Our results showed that all organic compounds change the soil *Trichoderma* spp. community structure. This is consistent with our previous study showing that some phenolic compounds, such as vanillin and vanillic acid, changed the cucumber rhizosphere soil *Trichoderma* spp. community structure (8,45).

Some species of *Trichoderma* spp. were able to degrade the sugars, phenolic compounds and other organic acids (7,36,41). Therefore, *Trichoderma* spp. might use *p*-hydroxybenzoic acid, glucose, succinic acid and glutamic acid as carbon resources in the soil environment. Previously, we found that vanillin and vanillic acid but not syringic acid changed the community structure and increased the abundance of cucumber rhizosphere soil *Trichoderma* spp. community (8,42,45). In this study, *p*-hydroxybenzoic acid but not *p*-coumaric acid increased the soil *Trichoderma* spp. community abundance. Thus, various phenolic compounds from cucumber play different roles in the interactions between the cucumber and *Trichoderma* spp. community.

The tested organic compounds had different influences on *Trichoderma* spp. community structure and abundance, suggesting that these various compounds play different roles in plant-microbial interactions. Similarly, previous studies have shown that various organic compounds had different effects on total soil total bacterial and fungal communities (11,31). Therefore, different components of root exudates have different selection effects on the whole soil microbial community and also perhaps on specific microbial taxa (such as *Trichoderma* spp.).

In agricultural ecosystems, continuous monocropping of same crop in the same land usually negatively affects the crop growth, a phenomenon known as 'soil sickness' (4,19,44,46,48). Accumulation of autotoxins, such as phenolic compounds, is one of the major causal agents of soil sickness (17,18,33). The structure and function of a soil microbial community are tightly linked and changes in microbial community's structure can alter its function (1,4,9,47). For example, *p*-coumaric acid could change the cucumber rhizosphere microbial community, which in turn exerts negative effects on the cucumber growth (52). In this study, *p*-coumaric acid increased the diversity but had no influence on the abundance of *Trichoderma* spp. community, indicating that *p*-coumaric acid promotes some taxa while inhibits other *Trichoderma* spp. However, this should be validated in future studies.

CONCLUSIONS

The exogenously added *p*-hydroxybenzoic acid, *p*-coumaric acid, glucose, succinic acid and glutamic acid changed the structure and increased the diversity indices of soil *Trichoderma* spp. community. *p*-Hydroxybenzoic acid, glucose, succinic acid and glutamic acid increased the soil *Trichoderma* spp. community abundance. Among all tested organic compounds, glucose had the strongest while *p*-hydroxybenzoic acid had the weakest influence on *Trichoderma* spp. community structure.

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