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Effect of benzoic acid on soil microbial communities associated with soilborne peanut diseases

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ABSTRACT

As potent allelochemicals, phenolic acids are believed to be associated with soilborne diseases, and can influence plant-microbe interactions. Benzoic acid (BA) is one major phenolic acid found in peanut (*Arachis hypogaea*) root exudates. The objectives of this study were to estimate the BA degradation in the soil and its effects on soil bacterial and fungal communities and to detect the specific taxa responding to BA amendment. BA degradation was investigated by monitoring the BA retained in the soil using high-performance liquid chromatography (HPLC) and the CO_2 production rate using gas chromatography (GC). The abundance and diversity of the bacterial and fungal communities were investigated by quantitative real-time PCR (qPCR) and Illumina MiSeq sequencing. The results showed that the BA concentration decreased significantly with an increased rate of CO_2 production during the first 36 h after amendment, implying that the BA in the soil was quickly metabolized by the microbes. Quantitative PCR analysis further detected a significant increase in soil bacterial and fungal abundances in response to BA addition, but a reduced bacteria-to-fungi ratio. As a result of BA amendment, the relative abundance of *Fusarium*, *Bionectria* and *Trichoderma* was markedly increased, whereas *Metarhizium* was reduced. Moreover, BA (0.1 mmol L^{-1}) promoted the mycelial growth, sporulation capacity and conidial germination of the peanut root rot pathogen *Fusarium* sp. *in vitro*. Among bacteria, the relative abundance of *Betaproteobacteria* was significantly increased in response to BA treatment, whereas the relative abundances of *AD3* and *Actinobacteria* were reduced. A deeper taxonomic analysis of the *Betaproteobacteria* taxa showed a great increase in the abundance of the genus *Burkholderia*, from 0.71% to 10.12%, in response to BA amendment. Constructing clone libraries with the partial 16S rRNA genes of *Burkholderia* further demonstrated that BA amendment had modified the *Burkholderia* species composition. Our results highlight that the effects of BA in the soil are reflected by changes in populations of soil microbes and suggest that the response of specific microbes such as *Fusarium* and *Burkholderia* to BA might be associated with the development of soilborne diseases in monocultures.

1. Introduction

In negative plant-soil feedback (NF), conspecifics make soil conditions less suitable for themselves (Bever et al., 2012; van der Putten et al., 2013). The prevalence of soilborne diseases in monoculture is a typical example of NF and has caused great reductions in crop yield (Huang et al., 2013). Due to industrial demand and farmer cultivation habits in China, intensive monoculture has increasingly occurred with certain cash crops such as soybeans (*Glycine max*), peanuts (*Arachis hypogaea*), watermelon (*Citrullus lanatus*), cucumbers (*Cucumis sativus*) and bananas (*Musa* spp.), which has resulted in severe occurrences of soilborne diseases (Li et al., 2014b; Ling et al., 2013; Wang et al., 2015; Zhou et al., 2014). To maintain yields, other practices, such as increasing pesticides and chemical fertilizers, have been applied, which has increased the input costs and constrained sustainable agricultural management; however, the mechanisms underlying the prevalence of soilborne diseases are not fully understood (Jordan et al., 2002; Wang et al., 2015; Zhou et al., 2014).

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As a major class of allelochemicals in the soil, phenolic acids are the primary polyphenols made by plants, and have multiple roles in plant-microbe interactions (Mandal et al., 2010; Singh et al., 1999). Studies have found that phenolic acids have toxic effects on the growth of plants and may be involved in the development of soilborne diseases (Baziramakenga et al., 1994; Inderjit Saini and Kaur, 2005; Bhowmik, 2004). In these studies, the effects of particular phenolic acids were primarily based on experiments without considering the soil microbial component, and high concentrations of active phenolic acids were often applied that may not be relevant to actual field situations (Bhowmik, 2004; Inderjit Saini and Kaur, 2005). When added to the soil, phenolic acids were rapidly metabolized by soil microorganisms and promoted the incidence of Fusarium wilt caused by *Fusarium oxysporum* in cucumber and watermelon (Blum, 1998; Wu et al., 2008; Ye et al., 2004; Zhou and Wu, 2012).

Peanut yield was generally decreased with increasing monocropping years, accompanied by the increasingly severe of soilborne diseases (Li et al., 2012; Li et al., 2014c; Wang and Chen, 2005). According to field investigation, yields of peanut were decreased by 28.9% and 51.2%, and root rot disease caused by *Fusarium* spp. increased from 8.1% to 17.4% and 54.9% in the 10 and 21 years monocropping fields, respectively, compared with the 3 years monocropping field. Various strategies, including crop rotation, application of chemical fungicides and organic manure amendments, have been proposed to alleviate the soilborne diseases (Jordan et al., 2002; Liu et al., 2015; Wang and Chen, 2005; Wang et al., 2015). Yet few research findings have demonstrated the successful increase of peanut yield and the control of peanut soilborne diseases under large scale field application conditions. During the root *Fusarium* infection, the concentration of phenolic acids in the root tissues and exudates was increased to inhibit the fungal procession (Lanoue et al., 2010; Michielse and Rep, 2009; Michielse et al., 2012). However, effects of phenolic acids on the soil suppressiveness towards peanut root rot disease still are not well understood.

Many soil microorganisms, including both bacteria and fungi, are reported to have pathways that degrade phenolic acids (Michielse et al., 2012; Pumphrey and Madsen, 2008). Using the plate counting method, the population of *F. oxysporum* in the soil was significantly increased in the presence of *p*-coumaric acid (Zhou and Wu, 2012). When *p*-coumaric acid or vanillic acid was artificially applied to soils, shifts in the microbial community of the rhizosphere were also detected using denaturing gradient gel electrophoresis (DGGE) or clone library methods (Zhou and Wu, 2012, 2013). Monoculture should dominate the development of soil microorganisms, due to a continuous release of phenolic acids into the soil. Associations between phenolic acids and peanut root rot disease need to be better understood in terms of soil microbial ecology (Li et al., 2014b; Zhao et al., 2015).

Intensive peanut monoculture in the hilly red soil regions of subtropical China has caused a significant decline in crop yield and quality and has increased susceptibility to root rot disease (Li et al., 2012; Liu et al., 2015; Wang and Chen, 2005). BA is one of the dominant phenolic acids in peanut root exudates, but its concentration is very low in peanut monoculture soil (Li et al., 2010, 2013, 2014a). We hypothesized that BA could be quickly metabolized by specific soil microbes and thus influence the composition of microbial community. The objectives of this study were as follows: (1), investigate the degradation of BA in the red soil; (2), evaluate its effects on the soil microbial community using high-throughput sequencing of the 16S rRNA and internal transcribed spacer 1 (ITS1) genes and to detect the specific taxa responding to BA treatment.

2. Materials and methods

2.1. Soil sampling

Soil was collected from a fallow agricultural field at the Ecological Experimental Station of Red Soil, Chinese Academy of Sciences, Yujiang County, Jiangxi Province, China (28°13′N and 116°55′E), where the monthly mean temperature varies from 5.9 °C in January to 30 °C in July. The annual precipitation is 1750 mm (averaged over 50 years). The selected field was previously planted with peanuts for three years and has then been kept fallow in the last five years.

Soil was collected from the surface layer (0–20 cm) and sieved (2 mm) to remove stones and plant residues. Some soil samples were air-dried to determine basic properties, and the others were stored at 4 °C until the experiments began. The soil was classified as Udic Ferrosol [FAO (1998) classification]. The soil had a pH_{H2O} of 4.78 and contained 7.63 g kg^{-1} of organic carbon, 0.83 g kg^{-1} of total nitrogen (N), 0.58 g kg^{-1} of total phosphate (P), 9.96 g kg^{-1} of total potassium, 18.82 mg kg^{-1} of available P, 8.54 mg kg^{-1} of NH_4-N, and 12.59 mg kg^{-1} of NO_3-N. BA was not detected in the soil.

2.2. Experiment 1: BA degradation in the soil

The experiment was arranged in a completely randomized design with three replicates that had the following treatments: soil amended with 1.2 μmol g^{-1} soil BA and control soil (CK) amended with distilled water. The soil was maintained at room temperature for at least 48 h prior to treatment with BA. A 12-mL aqueous BA solution (20 mmol L^{-1}) was added to 200 g of soil (dry weight equivalent) and mixed. Soil treated with distilled water served as the control. Soil moisture was adjusted to 40% of the maximum water holding capacity with distilled water. Ten grams of soil (dry weight equivalent) were placed into a 100 mL glass bottle and then incubated at 25 °C in the dark. Water loss was compensated for by adding distilled water daily. Bottles without soil were used as blanks.

Soils were sampled at 0, 24, 36, 48, 72 and 120 h of incubation and then stored at −25 °C until the determination of soil BA concentration. Four hours prior to gas sampling, a 100-mL syringe was used to refresh the air in the headspace of the bottles, which were then hermetically sealed with a rubber plug. The CO_2 production rate was monitored by analysing CO_2 concentrations in the headspace of the bottles.

2.3. Experiment 2: microbial response to BA

To examine the potential effects of BA and to detect the specific taxa responding to BA amendment on soil bacterial and fungal communities, BA was artificially supplied at two levels based on preliminary experiments and work by other researchers (Zhou and Wu, 2012). The experiment was arranged in a completely randomized design with three replicates. A 3-mL aqueous solution of 10 mmol L^{-1} or 20 mmol L^{-1} BA was added to 50 g of soil (dry weight equivalent) and mixed to obtain a low-level BA treatment (0.6 μmol g^{-1} soil, L) and a high-level BA treatment (1.2 μmol g^{-1} soil, H). Soil moisture was maintained at 40% of the maximum water holding capacity. Soil with an equal volume of distilled water was used as the control. Bottles were sealed with breathable sealing membranes and were incubated at 25 °C in the dark. Water loss was compensated for by adding distilled water daily. Soils were sampled on days 2 and 7 and stored at −25 °C and were used for microbiological analyses.