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Trichoderma communities in soils from organic, sustainable, and conventional farms, and their relation with Southern blight of tomato

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Abstract

The objectives of this research were to investigate the relationship between propagule numbers and genetic diversity of *Trichoderma* species and Southern blight of tomato caused by soilborne plant pathogen *Sclerotium rolfsii* in soils with long-term organic, sustainable, and conventional farms. Dilution plating was use to quantify the propagule numbers of *Trichoderma*, denaturing gradient gel electrophoresis (DGGE) and DNA sequence analysis were used to identify *Trichoderma* species, and greenhouse assay were conducted for soil suppressiveness to Southern blight. The propagule numbers of *Trichoderma* tend to be higher in soils from conventional farms. There was no clear separation for the propagule numbers of *Trichoderma* based on different management systems using canonical correspondence analysis (CCA). However, there was general separation for total microbial communities based on organic and conventional management systems using CCA. That suggests that the difference of soil suppressiveness to disease from organic, sustainable, and conventional farms is due to the difference of the total microbial diversity but not directly due to the *Trichoderma* populations in each farming system. The propagule numbers of soil *Trichoderma* did not significantly correlate with the diseases suppressiveness, although individual species of *Trichoderma harzianum* was shown to be related to disease suppressiveness. Moreover, several *Trichoderma* species were found in the soil tested based on DGGE and DNA sequence analysis. *Trichoderma hamatum*, *T. harzianum*, *Trichoderma virens*, and *Trichoderma erinaecem* were the most abundant species in tested soil.
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Keywords: *Trichoderma* spp.; Organic and conventional; Dilution plating; DGGE; DNA sequence analysis; Canonical correspondence analysis (CCA)

1. Introduction

Trichoderma is a genus which include species of free-living soil fungi, opportunistic, avirulent plant symbionts (Harman et al., 2004), asymptomatic endophytes (Wilberforce et al., 2003), and parasites of other fungi (Harman, 2006). It is often the major component of the mycoflora in soils of various ecosystems, such as agricultural farm soil, grassland, forest, marshes, deserts, and water (Danielson and Davey, 1973; Papavizas, 1985; Zhang et al., 2005). *Trichoderma* species possess high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, and capability to modify the rhizosphere (Woo et al., 2005). Moreover, *Trichoderma* species have long been recognized as agents for the control of plant pathogenic fungi, and have the ability of promoting plant growth and development (Samuels, 2006).

Sclerotium rolfsii Sacc. is a soilborne plant pathogen responsible for significant economic losses on a wide range of agronomic host plants that include 500 plant species in over 100 plant families (Punja, 1985). This disease can cause major loss to tomato (*Lycopersicon esculentum* Mill.) in Southern United States. The pathogen infects all portions of the plant in contact with the soil, and sclerotia can remain viable for many years and provide the primary inoculum for epidemics (Ristaino et al., 1991). Control of *Sclerotium* diseases is difficult and depends on a combination of chemical, cultural, and biological approaches

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(Mukherjee and Raghu, 1997). Early research showed that the higher propagule numbers of *Trichoderma* in soil resulted in the lower disease incidence of Southern blight of tomato in a controlled experiment (Bulluck and Ristaino, 2002), and the organically managed soils typically possessed the higher population of *Trichoderma* than the conventionally managed soils (Bulluck et al., 2002).

Organic production systems have increased in recent years in the United States (USDA, 2000). The transition from conventional to organic management practices could influence soil microbial diversity, microbial composition and subsequently change nutrient cycling and soil quality (van Diepeningen et al., 2006). Conventional farming systems characterized with extensive ploughing and application of synthetic fertilizers, fungicides, insecticides, and herbicides, which have been associated with loss of soil fertility, soil erosion, ground water pollution (Drinkwater et al., 1995), as well as the inhibition of the activity of soil microorganisms including soil fungi (Kennedy and Smith, 1995). Although many studies of microbial communities in soils from organic and conventional farming systems have been conducted (Bossio et al., 1998; Liu and Ristaino, 2003), there is little information on the responses of *Trichoderma* community to alterations of soil management practices, and on the relationship between the propagule numbers of *Trichoderma* or *Trichoderma* species and disease incidence of Southern blight of tomato in soils from organically and conventionally managed farms. Due to the ecological importance of *Trichoderma* species and their application as the biocontrol agents in agricultural fields, it is crucial to understand the changes in community structure of *Trichoderma* subjected to the transitions from conventional to organic agricultural management systems, and the relationship between the *Trichoderma* community and the incidence of Southern blight of tomato.

Identification of *Trichoderma* species is extremely difficult, due mainly to the few morphological characters available for differentiation of closely related species (Samuels, 2006; Rifai, 1969; Bissett et al., 2003). The advancement of molecular biology especially the sequence analysis of 18S rRNA and internal transcribed spacer (ITS) sequences has accelerated *Trichoderma* species classification and identification (Druzhinina and Kopchinskiy, 2006; Hagan et al., 2007). One of the molecular techniques widely used for community analysis is denaturing gradient gel electrophoresis (DGGE) (Muyzer et al., 1993). DGGE has been used to separate PCR product of the amplified 18S rDNA or ITS regions. The sequence analysis of purified PCR product amplified from excised DGGE bands is used to determine the phylogenetic placement of each individual band in the community. DGGE and DNA sequence analysis may give a more rapid and realistic view of soil *Trichoderma* diversity and their distribution in the agroecosystem. Such approaches may lead to a better understanding of soil *Trichoderma* community responsible for suppression of soilborne plant pathogens, and may eventually lead to improved and more reliable disease control.

The objectives of this research were to quantify the abundance of *Trichoderma* using dilution plating, and identify *Trichoderma* species using DGGE as well as DNA sequence analysis in soils from long-term organic, sustainable, and conventional farms. Multivariate statistical analysis was performed to sort out the relationship among the propagule numbers of *Trichoderma* or *Trichoderma* species, the incidence of Southern blight of tomato and soil physical, chemical, and biological factors.

2. Materials and methods

2.1. Soil sampling

Soil from 10 farms in North Carolina with a history of long-term organic, sustainable, or conventional crop production were sampled in August 2001, May 2002, and May 2003. Three of the farms were certified organic and did not use synthetic fertilizers or pesticides. They were located in Cedar Grove, NC (organic farm 1), Bear Creek, NC (organic farm 2), and Ivanhoe, NC (organic farm 3). Three of the farms sampled were classified as sustainable, meaning that synthetic pesticides were not used; however, synthetic fertilizers were used. These farms were located in Graham, NC (sustainable farm 1), Bear Creek, NC (sustainable farm 2), and Clinton, NC (sustainable farm 3). Four conventional farms were sampled. These farms used monoculture, synthetic fertilizers, pesticides, and herbicides. These farms were all located in or near Clinton, NC (conventional farms 1–3) or in Faison, NC (conventional farm 4). Details of the soil texture, years under current farming systems, crops in the field, pesticides, and soil fertility amendments are shown in Supplementary Table S1.

Soil samples from each farm were collected in the location where the homogeneous crops were grown. Three composite soil samples were collected from each of the 10 farms in the fall of 2001, and late spring of 2002 and 2003. Total 20 kg of soil was removed from three contiguous areas at each farm using a 25 mm soil auger in a serpentine pattern down each row to a depth of 20 mm and bulked. Two undisturbed soil cores were removed from each of the three locations at each farm for bulk density and water release measurements. Composite soil samples were stored in coolers on ice until returning to lab. For soil dilution plating, the soils were transferred to a storage room and stored at 4 °C until the time of analysis; for DGGE analysis, the soils were stored at −20 °C freezer until the time of analysis.

2.2. Soil physical characters

Undisturbed soil cores collected from the field with soil sampling rings of known volume at each farm were weighed and then dried in an oven and reweighed for bulk

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