European Journal of Soil Biology 45 (2009) 356–362

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Effect of cinnamic acid on soil microbial characteristics in the cucumber rhizosphere

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ARTICLE INFO

Article history:
Received 23 June 2008
Received in revised form
25 March 2009
Accepted 2 April 2009
Available online 17 April 2009
Handling editor: Petra Marschner

Keywords:
Allelochemical
Cucumber
Soil microbial ecology

ABSTRACT

In order to elucidate the effect of allelochemicals on soil microbial characteristics in the cucumber rhizosphere, the soil microbial biomass and respiration, community functional diversity and RAPD marker diversity as affected by exogenous cinnamic acid were studied. Exogenous cinnamic acid increased soil microbial respiration and the metabolic quotient, but decreased soil microbial biomass-C. Soil microbial community functional diversity and genetic diversity (as indicated by RAPD markers) were also significantly altered by exogenous cinnamic acid. These results suggest that allelochemicals can change soil microbial genetic diversity, biological activity and microbial metabolic activity, which alter soil microbial ecology and accordingly affect the growth of cucumber with accumulation in the soil of allelochemicals.

1. Introduction

It is well known that continuous cropping can affect crop growth and decrease yield, quality and disease resistance [41,40]. Continuous cropping obstacles are usually found in agricultural crops, especially in horticultural crops [16,39,50], and are attributed to the buildup of pests, soil physiochemical property disorders, autotoxicity and soil microbes [50,20,52,40]. Hu et al. [22,21] observed that continuous cropping of cucumber disturbed the soil ecological balance and decreased soil microbial community diversity. Studies on agricultural soils have reported that monoculture systems, compared to multi-cropping systems, have decreased soil microbial biomass-C and N, potential cumulative N mineralization, enzyme activities, microbial community functional diversity and genetic diversity [17,29,1,49,47]. Similarly, the microbial diversity in mixed forests soil was higher than that in simple forests [12,56]. The negative effect of continuous cropping may be due to allelochemical effects on soil microbial ecology [27].

Some plant species have allelopathic potential by releasing allelochemicals into the rhizosphere [36,52]. Allelochemicals affect plant metabolism such as photosynthesis, respiration, and ion uptake [4,13], and change the chemical and biological characteristics of the rhizosphere [9]. Previous studies reported that cucumber plants possess allelopathic potential by exuding allelochemicals such as benzoic and cinnamic acids, among which cinnamic acid is a widespread and common example [52]. The released allelochemicals also serve as an important carbon and energy source for microorganisms in the rhizosphere [10,9]. However, the influence of allelochemicals on microbial community structure remains largely unknown.

In the present study, we applied a single allelochemical to soil, which clearly differs from the effects of the commercial practice of continuous cropping of cucumber, and due to restrictions in the method of detecting allelochemicals in soil, we didn't analyze cinnamic acid content in soil during the course of the experiment. Allelochemical interactions are complex and integrate biochemical and ecological processes so future investigations will require a multidisciplinary approach.

Many previous studies of autotoxic effects have mainly focused on the plant itself. There is little information about the effect on soil microbes, even though soil microbes play a key role in soil–plant interactions. One possible reason is the difficulties involved in the separation and identification of soil microbial community. It is estimated that the microbes that have been identified so far comprise no more than 10% of the actual number. Moreover, the methods of separation and identification lack comparability [18,38]. The interaction between microbes and soil granules has also limited quantitative analyses [2,37].

Cucumber is one of the major greenhouse vegetables in China and its autotoxic effect is very strong [51]. The allelopathic potential of cucumber was reported based on the suppressive effect on the

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1164-5563/$ – see front matter
doi:10.1016/j.ejsobi.2009.04.001

Table 1
Basic physical and chemical properties of test soil.

O.M. (%)	Total N (%)	Alkaline N (mg kg^{-1})	Total P (%)	Avai. P (mg kg^{-1})	Slowly Avai. K (mg kg^{-1})	Avai. K (mg kg^{-1})	pH	EC (Ms cm^{-1})
3.67	0.239	141.1	0.138	260.7	901.5	150.3	6.99	293

growth of some weed species [35,3]. Yu and Matsui [54] found that uptake of SO_4^{2-} and Mg^{2+} was significantly inhibited even at 0.01 mmol mL^{-1} cinnamic acid, whereas the uptake of NO_3^-, K^+ and Ca^{2+} was significantly inhibited at a high cinnamic acid concentration (0.1 mmol mL^{-1}). Lockerman and Putnum [31] confirmed that certain cucumber accessions strongly inhibited the growth of some weeds under controlled conditions. Autotoxicity in cucumber plants was regarded as the result of the accumulation of phytotoxic compounds [34]. In this study, soil microbial biomass and respiration, community functional diversity and genetic diversity in the cucumber rhizosphere, as affected by exogenous cinnamic acid, were analyzed. The objective was to determine the effect of cinnamic acid on different aspects of soil microbial ecology, and to understand the mechanism that makes cucumber continuous cropping problematic.

2. Materials and methods

2.1. Plant material and soil

Seeds of cucumber cv. 'Changchun Mici' (obtained from the Seed Company of Northeast Agricultural University) were washed with sterilized water. The seeds were germinated in the dark at 28 °C for 2 d. After germination the seedlings were grown in a 9.5 h light/14.5 h dark photoperiod. The seedlings were transplanted into pots (25 cm long × 25 cm wide × 30 cm high) containing soil in which cucumber had never been planted. The soil was obtained from the experimental station of Northeast Agricultural University, Harbin, Heilongjiang province (45°41′N, 126°37′E). The basic physical and chemical properties of the tested soil are presented in Table 1 [25].

2.2. Treatment with cinnamic acid

In a previous report, under solar greenhouse conditions, the levels of *p*-hydroxybenzoic acid, ferulic acid and benzoic acid in the soil increased with increasing duration of continuous cropping and were obviously higher after continuously cropping for 5, 7 and 9 years than for 1 and 3 years. The level of allelochemicals in soil after 9 years of continuous cropping reached 47.93 μg g^{-1} soil [32]. In commercial practice, continuous cropping usually exceeds 20 years and due to restrictions in the method of detecting allelochemicals in soil, the actual content of allelochemicals in soil may be higher than the above concentration. So, in the test, we used cinnamic acid with the concentrations of 25, 50, 100 and 200 mg kg^{-1} soil.

Cinnamic acid (Wantai Biological Technology Company) was dissolved in 95% ethanol. Solutions comprising four concentrations (25, 50, 100 and 200 mg kg^{-1} soil) of cinnamic acid were prepared using the same volume of ethanol. Seven days after transplantation of the cucumber seedlings, 25 mL cinnamic acid solution was applied to the soil in each pot (except the control). Three replicates of each treatment were used. The seedlings were placed in a greenhouse (25 °C, 4000 lux, with a 9.5 h day/14.5 h night cycle, and 75% relative humidity) using a completely randomized design. Soil without cinnamic acid was used as a control. The cucumber seedlings were watered by drip irrigation and at the bottom of each pot, plastic film was used to prevent leaching. After 30 d cultivation, the soil in each pot was harvested. One part of each sample was used to determine the soil microbial biomass-C and soil microbial community functional diversity. The remaining soil was stored at −70 °C and was used to determine the soil microbial community DNA sequence diversity.

2.3. Soil microbial biomass-C and basal respiration

The fresh soil samples were sieved (pore size < 2 mm) and large pieces of plant roots were removed. The soil samples were incubated for 7 d at 25 °C and the moisture contents were adjusted to 50% of their water-holding capacity prior to microbial biomass and respiration measurements. A chloroform fumigation-extraction method was used to determine the soil microbial biomass-C. The content of organic C was determined by an automated TOC analyzer (Shimazu, TOC-500) [19]. The basal respiration (CO_2 evolution) was measured in 500 cm^3 soil jars using gas chromatography to measure the headspace CO_2 that accumulated over 24 h at 25 °C from 50 g fresh soil.

2.4. Extraction and purification of soil microbial DNA

Microbial community DNA in the soil was extracted using the SDS/CTAB method described by Jiao et al. [26]. Extracted DNA was purified with the Promega Wizard Kit (Promega Co.).

2.5. Amplification and RAPD analysis of soil microbial DNA sequence diversity

The DNA sequence diversity of the soil microbial community was evaluated by Random Amplified Polymorphic DNA (RAPD) analysis. We used random primers to amplify the microbial community DNA from the soil samples [48]. Since primers sequences were random and non-selective to DNA samples, amplification for one primer was equal to one random sampling from the whole microbial DNA sequences. The number of RAPD fragments was considered to represent the RAPD fragment richness of the whole DNA sequences. Ten random primers (supplied by Beijing Baotaike Co. and Shanghai Sangon Co.), which were selected from 100 primers and that amplified fragments clearly, were used to amplify soil microbial DNA in the soil samples. The selected primers and the nucleotide sequence of each are listed in Table 2.

The most suitable RAPD conditions were determined using the method of Jiao et al. [26]. Amplification using the polymerase chain reaction (PCR) was performed in a 25 μl total volume containing 1 × PCR buffer, 7 ng target DNA, 20 pmol random primers, 1.5 unit Taq DNA polymerase, 3.0 mM $MgCl_2$ and 0.2 mM dNTPs. DNA amplification was carried out in a Perkin–Elmer 9600 thermocycler with the following procedure: an initial denaturing step at 94 °C for 3 min; 40 cycles for 1 min at 94 °C (denaturation), 40 s at 37 °C

Table 2
The selected primers and their nucleotide sequence.

Code	Sequence 5′ → 3′	Content of GC (%)	Code	Sequence 5′ → 3′	Content of GC (%)
1215	ACACTCTGCC	60	Q8	CCTCCAGTGT	60
1268	CACCGATCCA	60	V3	CTCCCTGCAA	60
1508	AAGAGCCCTC	60	A12	TCGGCGATAG	60
236	ACACCCCACA	60	M12	GGGACGTTGG	70
261	CTCAGTGTCC	60	1387	CTACGCTCAC	60