Soil Biology and Biochemistry 125 (2018) 64–74

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Manipulation of microbial community in the rhizosphere alleviates the replanting issues in *Panax ginseng*

Linlin Dong[a,1], Jiang Xu[a,1], Yong Li[b,1], Hailan Fang[a], Weihao Niu[a], Xiwen Li[a], Yujun Zhang[a], Wanlong Ding[b], Shilin Chen[a,*]

[a] Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, 100700, China
[b] Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, 100193, China

ARTICLE INFO

Keywords:
Allelochemicals
Autotoxicity
Continuous cropping
Soil bacteria
Sphingobacterium sp.
Replanting disease

ABSTRACT

The replanting ginseng (*Panax ginseng* C. A. Meyer) in soil where ginseng has previously been grown usually fails due to issues associated with continuous cropping. Successful replanting requires a rotation cycle of more than 30 years. Crop failure at replant sites has been attributed to the accumulation of toxic substances in soil and changes in the rhizosphere microbiome. Soil acidification, toxic compound accumulation, and decreased bacterial diversity were detected in soils during ginseng cropping. The accumulation of toxic diisobutyl phthalate (DiBP) is negatively related to the abundance of *Arthrobacter*, *Burkholderia*, *Rhodanobacter*, and *Sphingobacterium*. A total of 153 strains were isolated from the rhizosphere soil of ginseng seedlings and identified as DiBP-degrading bacteria. Among these strains, *Sphingobacterium* sp. PG-1 degraded more than 90% of DiBP within 72 h. The abundance of PG-1 decreased by 79.9% in soils that were cropped with ginseng for three years. DiBP content decreased by 39.2%, and the ginseng death rate was decreased by 40.1% after replanting ginseng in soils inoculated with PG-1. Results revealed that the reduced abundance of DiBP-degrading microbes resulted in the accumulation of toxic compounds that disrupted the microbial ecology. This study provides insights into the integrated mechanism underlying replanting problems in terms of the chemical, biological, and genetic make-up of ginseng. Manipulating soil microbial communities is an effective strategy to alleviate ginseng replanting problems and increase crop productivity.

1. Introduction

Ginseng (*Panax ginseng* C. A. Meyer), which belongs to the family Araliaceae, presents anti-inflammatory and antitumor effects, and it is commonly used in traditional Chinese medicine (Choo et al., 2008; Ernst, 2010). The current global market value of this species is approximately $3.5 billion annually (Hong et al., 2006). In general, wild ginseng typically grows in the mountainside, where it is humid, the soil is fertile, and it is partially covered from extreme sunlight by lush vegetation (Zhang et al., 2008a). Wild ginseng has been exhausted, and has been gradually replaced by cultivated ginseng. At present, ginseng production is mainly dependent on cultivation in China, Korea, and Japan (Jung et al., 2014). Deforestation with subsequent ginseng planting is the common cultivation pattern in the Changbai Mountain region of China. Ginseng is harvested 4–5 years after sowing, and subsequent replanting commonly fails due to some problems associated with continuous cropping (Ying et al., 2012). Replanting adversely affect the crop quality and yield, and render the soil inappropriate for ginseng cultivation for up to 30 years (Li et al., 2011). This need for cropping discontinuity is promoting large-scale deforestation, with an average of 7000 hm^2 of forest being cut-down every year in Jilin province, the main ginseng production region of China (Yang et al., 2004; Xiao et al., 2016). To conserve forest resources and maintain the ecology of the forest environment, strategies to overcome the replanting problem in ginseng cultivation are needed.

Replanting problems affect many crops worldwide and result in severe losses in forestry and agriculture (Li et al., 2010a; Mazzola and Manici, 2012; Wu et al., 2015). Multiple factors, including the outbreak of soil-borne diseases, deterioration of soil physico-chemical properties, allelopathy/autotoxicity, and changes in soil microbial community composition have been implicated in a number of replanting problems (Ogweno and Yu, 2006; Li et al., 2011; Huang et al., 2013). Allelochemical compounds can influence the microbial species, thereby increasing or decreasing the number of soil-borne disease in continuous

* Corresponding author. No.16 Nanxiaojie, Dongzhimennei Ave., Beijing, 100700, China.
E-mail address: slchen@icmm.ac.cn (S. Chen).
[1] These authors contributed equally.

https://doi.org/10.1016/j.soilbio.2018.06.028
Received 16 January 2018; Received in revised form 28 June 2018; Accepted 28 June 2018

cropping systems (Huang et al., 2013; Wu et al., 2015). *p*-coumaric acid in cucumber root exudates promotes the growth of soil-borne pathogens (Zhou and Wu, 2012). Allelopathic potentials of toxic substances, such as benzoic acid, diisobutyl phthalate (DiBP), palmitic acid, *p*-hydroxybenzoic acid, and cinnamic acid, have been detected in ginseng-planted soils; these substances inhibit the growth of ginseng seedlings (Li et al., 2011). Xiao et al. (2016) have reported that the key factors for discontinuous cultivation of ginseng are imbalance in microbial communities and the outbreak of soil-borne diseases caused by the accumulation of ginseng root exudates. These results suggest that multiple aspects of the biological and chemical properties of soil are negatively altered in the ginseng continuous cropping system and contribute to the replanting problem. Hence, studying the integrated factors can contribute to solving problems in continuous cropping of ginseng.

Biotic and abiotic factors, such as plant species, soil chemical properties, and cropping systems, influence the structure and diversity of microbial communities in the rhizosphere (Lauber et al., 2008; Berg and Smalla, 2009; Fierer et al., 2012; Dong et al., 2017), and the presence of beneficial and harmful groups can significantly affect soil fertility, plant growth and crop quality (Garbeva et al., 2004; Nayyar et al., 2009; Wu et al., 2011). Continuous monoculture systems also result in soil microbial communities in which the abundance of beneficial bacteria is depleted and the abundance of pathogenic microbes is enhanced. This effect has been observed for other herbs used in traditional Chinese medicine, including Chinese foxglove (*Rehmannina glutinosa* (Gaertn.) Steud.) (Wu et al., 2015) and false starwort (*Pseudostellaria heterophylla* (Miq.) Pax) (Zhao et al., 2016), and tends to become more pronounced with time. Perturbations to microbial community structure often result in changes to specific microbial activities and one of the most important soil microbial functions with respect to ginseng cultivation is the degradation of allelochemicals, especially phenolics. Several types of bacteria are able to degrade plant-derived phenolics, for example a strain of *Pseudomonas aeruginosa* (MTCC 4996) degrades phenol up to a concentration of 1300 mg L^{-1} within 156 h (Kotresha and Vidyasagar, 2008), and an isolate of *Bacillus methylotrophicus* obtained from the rhizosphere of cucumber (*Cucumis sativus* L.) has been shown to have considerable efficacy with respect to the degradation of ferulic acid (Zhang et al., 2015). Although allelochemical compounds accumulate with plant development in the rhizosphere (Zhao et al., 2015), the decrease or absence of toxin-degrading microbes is also one of the main causes for the accumulation of toxic substances. We hypothesized that 1) the problem associated with replanting ginseng is a combination of soil biological and chemical properties, and 2) one of the main factors causing replanting problem is the accumulation of toxic substances due to the decrease or absence of special toxin-degrading bacteria as a result of the continuous cropping of ginseng.

This study aims to provide a mechanistic understanding of the replanting problem associated with the continuous cropping of ginseng and to identify effectively improving strategies. We have examined the following: 1) changes in soil chemical and biological properties in continuous cropping fields of ginseng, 2) relationship between the populations of special microbial taxa and the accumulation of toxic substances, 3) screening and identification of toxin-degrading bacteria in the soils in which ginseng had been cultivated, 4) changes in the relative abundance of toxin-degrading bacterial populations in the continuously cropped soils of ginseng, and 5) efficacy of toxin degradation following the addition of toxin-degrading bacteria to replant soils.

2. Materials and methods

2.1. Site description and soil sampling

Our study area is located in Fusong, Jilin Province (127°17′N, 42°26′E, average altitude: 512 m), which is the main region for ginseng production. The area is a secondary forest, with more than 30 years of growth after timber harvest without the use of herbicides, tillage, or chemical fertilizers. The vegetation in this region was primarily composed of *Pinus koraiensis*, *Larix gmelinii*, *Quercus mongolica*, *Tilia tuan*, *Acer mono*, *Spiraea salicifolia*, and *Corylus heterophylla*. The area of our plantation has a typically continental climate with an average annual rainfall of 800 mm. The annual mean temperature is 4.3 °C, the mean annual sunshine hour is 2200–2500 h (Chen et al., 2010) and the soil is classified as Chernozem.

Ginseng plants are commonly grown in mountain forests after deforestation. Ginseng is cultivated according to regional practices and followed the standard operating procedures of Good Agricultural Practice (Heuberger et al., 2010; Zhang et al., 2010). Two-year old uniform ginseng seedlings were transplanted into new forest soils and cultivated for a further 3 years until harvest. This procedure is the main cultivation pattern in ginseng-producing regions. Our plantation comprised three replicate blocks, and each block contained four plots (1.5 m × 20 m) for each treatment. Ginseng seedlings after transplantation were randomly cultivated for 1, 2, or 3 years in each plot in one block, and soil samples from ginseng-cultivated land were referred to as GL1, GL2, and GL3, respectively. One of the four plots in each block, a forest land (FL) without cultivation of ginseng seedlings, served as control. Six ginseng seedlings were randomly selected from each plot. Rhizosphere soil adhering to ginseng roots was collected by brushing the roots, and soil samples from each plots were pooled together as one sample. Control samples from six cores (5 cm in diameter and 20 cm in depth) were randomly selected from each FL plot and combined into one sample. We hypothesized that the changes in chemical and biological properties occurred in continuously cropped soils compared with those in natural FLs, and they could cause replanting problems. Soil samples collected from FLs acted as control. Twelve samples were collected, homogenized by passing through a 2 mm sieve, and divided into two subsamples. A subsample was used for soil property analysis, and the other one was stored at −80 °C for further processing.

2.2. Soil physical and chemical characteristics

Soil pH was measured using a soil-to-water ratio of 1:2.5 (w/v). Total nitrogen (N) was determined using an elemental analyzer (Vario EL III, Germany). Soil organic carbon (C) was measured through the $K_2Cr_2O_7$ oxidation–reduction titration method by Page et al. (1982). Soil available phosphorus (AP) was determined using the Mo–Sb colorimetric method (Gyaneshwar et al., 2002). Soil available potassium (AK) was measured according to the method of McLean and Watson (1985). Soil particle size distribution was analyzed by laser diffraction using a Longbench Mastersizer 2000 (Malvern Instruments, Malvern, UK). Soil particles were categorized as clay (0–2 μm), silt (2–50 μm), and sand (50–2000 μm) according to the soil texture classification system of the US Department of Agriculture. Soil particle size distribution was summarized as the volume fractal dimension value to control the number of variables used in structural equation modeling (Hu et al., 2013). Phenolic compounds from soil extracts were identified through gas chromatography–mass spectrometry (GC–MS, Agilent Co., Ltd.; HP-5^{ms}, equipped with a 0.25 mm × 30 m × 0.25 μm capillary column) with He as the carrier gas (Li et al., 2011). Briefly, each sample was extracted with methanol (v/w = 5:1) for 24 h at 150 rpm. Soil extracts were filtered, vaporized, dried under a stream of N, and then dissolved in 5 mL of methanol (Sigma-Aldrich, St. Louis, MO, USA). The injection temperature, transfer line, and ion source were heated to 250 °C, 270 °C, and 200 °C, respectively. The temperature program for each sample is as follows: initial temperature was set at 60 °C for 1 min, increased to 180 °C at a rate of 10 °C min^{-1} and 280 °C at a rate of 15 °C min^{-1}, and held for 15 min. Detection was achieved by mass selective detection in scan mode. The identities of chemical compounds from all samples were confirmed by searching the mass spectral database based on their GC–MS response compared with the mass spectra and retention time. Certain compounds were quantified according to the standard of known concentration using the method