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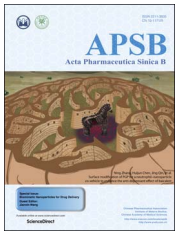

ORIGINAL ARTICLE

Rhizospheric microbial communities are driven by *Panax ginseng* at different growth stages and biocontrol bacteria alleviates replanting mortality

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Abstract The cultivation of *Panax* plants is hindered by replanting problems, which may be caused by plant-driven changes in the soil microbial community. Inoculation with microbial antagonists may efficiently alleviate replanting issues. Through high-throughput sequencing, this study revealed that bacterial diversity decreased, whereas fungal diversity increased, in the rhizosphere soils of adult ginseng plants at the root growth stage under different ages. Few microbial community, such as *Luteolibacter*, Cytophagaceae, *Luteibacter*, *Sphingomonas*, Sphingomonadaceae, and Zygomycota, were observed; the relative abundance of microorganisms, namely, *Brevundimonas*, Enterobacteriaceae, *Pandoraea*, Cantharellales, *Dendryphion*, *Fusarium*, and Chytridiomycota, increased in the soils of adult ginseng plants compared with those in the soils of 2-year-old seedlings. *Bacillus subtilis* 50-1, a microbial antagonist against the pathogenic *Fusarium oxysporum*, was isolated through a dual culture technique. These bacteria acted with a biocontrol efficacy of 67.8%. The ginseng death rate and *Fusarium* abundance decreased by 63.3% and 46.1%, respectively, after inoculation with *B. subtilis* 50-1. Data revealed that microecological degradation could result from ginseng-driven changes in rhizospheric microbial communities; these changes are associated with the different ages and developmental stages of ginseng plants. Biocontrol using microbial antagonists alleviated the replanting problem.

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1. Introduction

Panax ginseng C.A. Meyer demonstrates neuroprotective effects against ischemic stroke[1]. Ginseng plants are mainly distributed in Asia, particularly in China and South Korea[2]. The current annual global market value of this species is approximately 3.5 billion dollars[3]. Wild ginseng resources have dwindled because of excessive and predatory exploitation; thus, wild ginseng has been gradually substituted with cultivated ginseng in the mainstream market[4,5]. Ginseng is continuously cultivated in fixed plots for 4–5 years; however, subsequent replanting commonly fails because of obstacles to continuous cropping[6]. Decades of crop rotation are needed for successful replanting. The replanting issue is a severe drawback that hinders the development of the ginseng industry and thus requires urgent resolution.

The replanting problem is caused by the deterioration of soil physicochemical properties, allelopathy/autotoxicity, outbreak of soil-borne diseases, and changes in soil microbial communities[7–9]. The change in soil microbial community is a major factor that hinders crop replantation[10,11]. Imbalances in rhizospheric microbial communities disrupt ginseng cultivation[12]. Microbial communities change during ginseng cultivation[13], and the increased abundance of pathogenic microorganisms is related to the occurrence of soil-borne disease[14]. Collective changes in the rhizospheric microbial community may cause replanting issues.

Plants of different ages can alter microbial community[15]. The continuous cropping of *Panax quinquefolius* L. changes the microbial community in arable soil[16]. Ginseng plants of different ages drive changes in microbial community. Specifically, rhizospheric and nonrhizospheric soil microbial communities in a particular site become drastically different with ginseng growth[4]. The diversity and relative activity of soil microbial communities change throughout plant development[17]. However, the mechanism through which *Panax* plants of different ages and developmental stages mediate microbial community is unclear.

Root rot is a severe disease that hinders the replantation of *Panax* plants[18]. *Fusarium oxysporum* is the main pathogenic fungus of root rot in *Panax* plants[14,19]. The relative abundance of *F. oxysporum* increases with notoginseng growth and is significantly related with the death rate of ginseng seedlings[14]. The application of biocontrol bacteria could effectively alleviate the occurrence of root rot. Biological control using microbial antagonists has attracted interest as an effective method to decrease the abundance of plant pathogens due to its nontoxic nature[20]. Biocontrol bacteria have important roles in plant defense, and many isolates have shown antagonistic activity against phytopathogenic fungi[21]. In tomato, *Bacillus amyloliquefaciens* RWL-1 inhibits the growth of *F. oxysporum*[22]. Nevertheless, microbial antagonists against ginseng root rot are rare.

Herbgenomics has been utilized in recent investigations on medicinal plants. It involves the use of genomic tools, including metagenomic sequencing technology, to facilitate the analysis of rhizospheric microecology[23]. In the present study, 16S and 18S rRNA genes were analyzed through high-throughput sequencing to illustrate the changes in microbial diversity and composition in the rhizosphere soil of ginseng seedlings at different ages and developmental stages. Furthermore, biocontrol bacteria against *F. oxysporum* were isolated through a dual culture technique, and their inhibitory activity against the target pathogen in replanting soil was confirmed. The results of this study provide insight into the reasons that underlie the replanting issues caused by rhizospheric microbial communities. These data may provide an effective soil bioremediation method to replanting issues associated with Chinese medicinal plants.

2. Materials and methods

2.1. Field experiment and soil extraction

The field experiment was performed in a ginseng plantation in Jingyu, Jilin Province (42°20′N, 126°50′E, 775 m a.s.l.), the main ginseng-producing region in China. This region has a northern temperate continental climate and receives an annual precipitation of approximately 767 mm. The plough layer in the plantation consists of gray-brown soil.

Disease occurrence and mortality rates of ginseng seedlings generally increase after 2 years of consecutive growth. Thus, we analyzed the influence of 2-, 3-, and 4-year-old transplanted seedlings on rhizospheric microbial communities. 2-, 3-, and 4-year-old ginseng seedlings were transplanted in each plot in our plantation and denoted as 2-y, 3-y, and 4-y, respectively. Field plots were arranged following a completely randomized block design, with 3 replicate plots (1.7 m × 8.0 m) per plant age. Ginseng was cultivated strictly in accordance with the standard operating procedures of good agricultural practice[24,25]. The distinct stages of ginseng development are as follows: vegetative, flowering, fruiting, root growth, and annual dormancy (Supplementary Information Table S1). During dormancy, the aboveground parts of ginseng wither and underground root activities weaken. Thus, soil samples obtained during this stage were excluded from analyses.

This experiment included 36 soil samples that were obtained from 2-, 3-, and 4-year-old ginseng seedlings at 4 developmental stages, namely, vegetative (2-Ve, 3-Ve, and 4-Ve), flowering (2-Fl, 3-Fl, and 4-Fl), fruiting (2-Fr, 3-Fr, and 4-Fr), and root growth (2-Ro, 3-Ro, and 4-Ro). Six ginseng seedlings were randomly collected from each plot (1.7 m × 8.0 m). Roots were shaken free of soil, and rhizosphere soil fractions were brushed and pooled into one sample. Soil samples were obtained from 3 replicates per treatment and were homogenized by passing through a 2 mm sieve prior to further processing. The soil characteristics are described in Supplementary Information Table S2.

2.2. DNA extraction and PCR amplification

Total soil DNA was extracted from 0.1 g of freeze-dried soil using a MoBio Powersoil Kit (MoBio Laboratories Inc., Carlsbad, CA, USA) in accordance with the manufacturer's instructions. Then, 16S and 18S rRNA gene fragments from each sample were amplified using the conserved primers 27F/338R[26] and 817F/1196R[27], respectively. The forward and reverse primers contained an eight-base pair barcode (Supplementary Information Table S3). Amplification and purification were performed as previously described[28]. Purified PCR products were quantified with Qubit®3.0 (Life Invitrogen, Germany). The amplicons were pooled in equimolar ratios for sequencing.

2.3. High-throughput sequencing

The pooled DNA product was paired-end sequenced (2 × 250) on an Illumina HiSeq platform (Shanghai Biozeron Co., Ltd., China) following standard protocols. Raw FASTQ files were demultiplexed and quality filtered using QIIME with the following

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