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Research article

Effects of cultivation ages and modes on microbial diversity in the rhizosphere soil of *Panax ginseng*

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ABSTRACT

Background: *Panax ginseng* cannot be cultivated on the same land consecutively for an extended period, and the underlying mechanism regarding microorganisms is still being explored.
Methods: Polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE) and BIOLOG methods were used to evaluate the microbial genetic and functional diversity associated with the *P. ginseng* rhizosphere soil in various cultivation ages and modes.
Results: The analysis of microbial diversity using PCR-DGGE showed that microbial communities were significantly variable in composition, of which six bacterial phyla and seven fungal classes were detected in *P. ginseng* soil. Among them, Proteobacteria and Hypocreales dominated. *Fusarium oxysporum*, a soilborne pathogen, was found in all *P. ginseng* soil samples except R0. The results from functional diversity suggested that the microbial metabolic diversity of fallow soil abandoned in 2003 was the maximum and transplanted soil was higher than direct-seeding soil and the forest soil uncultivated *P. ginseng*, whereas the increase in cultivation ages in the same mode led to decreases in microbial diversity in *P. ginseng* soil. Carbohydrates, amino acids, and polymers were the main carbon sources utilized. Furthermore, the microbial diversity index and multivariate comparisons indicated that the augmentation of *P. ginseng* cultivation ages resulted in decreased bacterial diversity and increased fungal diversity, whereas microbial diversity was improved strikingly in transplanted soil and fallow soil abandoned for at least one decade.
Conclusion: The key factors for discontinuous *P. ginseng* cultivation were the lack of balance in rhizosphere microbial communities and the outbreak of soilborne diseases caused by the accumulation of its root exudates.

1. Introduction

Panax ginseng Meyer (Araliaceae), one of the most well-known Chinese herbal medicines, was formerly a wild plant grown in the northeastern region of China. Generally, *P. ginseng* is mainly dependent on artificial cultivation in China, and it is also cultivated in Korea and Japan [1]. However, the yield is severely hindered by continuous cropping obstacles of *P. ginseng*. In brief, *P. ginseng* cannot be cultivated on the same plot of land consecutively for several years or even decades owing to high requirements for soil quality [2]. Continuous cropping obstacles of *P. ginseng* always make its roots turn rusty and rot on account of soilborne diseases [3]. A large-scale deforestation is increasingly sharpening owing to discontinuous cultivation that not only damages forest resources but is also a limiting bottleneck on the sustainable development of *P. ginseng* crops. Hence, the contradiction between *P. ginseng* industries and the forest industry has become a major technical problem that needs to be solved urgently. Accumulating lines of evidence indicate that four major factors—deterioration of soil physicochemical characteristics, outburst of soilborne diseases, imbalance of soil microbial community, and autotoxicity of *P. ginseng*—can result in discontinuous cultivation of *P. ginseng*

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[4,5]. In the past several years, although a few researchers have carried out investigations on soil improvement and sterilization, the underlying mechanisms responsible for the relationship between microbial diversity and discontinuous cultivation are still poorly understood.

Increasing evidence indicates that rhizosphere microorganisms play a vital role in nutrient cycling, organic matter decomposition, and the maintenance of soil fertility [6]. In addition, the soil microbial community is also an important bioindicator of soil function [7]. Therefore, many investigations on discontinuous cultivation were focused on the evaluation of soil quality and the microbial community. Interestingly, several findings from previous studies have demonstrated that continuous farming resulted in an imbalance in soil ecology and alterations of microbial diversity in rhizosphere soil [8,9]. Although a number of microbial strains (comprising $< 1\%$ in total) have been isolated from successive cultivation soil [10–12], most of the microbial communities and their composition in rhizosphere soil are still difficult to analyze. The availability of modern tools in microbial ecology have permitted the study of microbial communities related to plant growth and development, *in situ* localization of important forms, and the monitoring of microbes when their quantities change in the soil environment [13].

In the present study, molecular culture-independent methods based on 16S rDNA and 18S rDNA gene diversity [14,15], polymerase chain reaction and denaturing gradient gel electrophoresis (PCR-DGGE), and random amplified polymorphic DNA were successfully used to examine the microbial community and dynamics of dominant microbial species in plant rhizosphere soil during its growth [4,16]. Compared with the traditional analysis method, PCR-DGGE has been one of the most widely used tools to assess the structure of microbial communities in soil and to determine the community dynamics in response to soil and other variations both quickly and economically [17,18]. Moreover, soil functional diversity was commonly used as an indicator for soil quality, and studies on the relationship between the rhizosphere microbial structure and function have been becoming a hot topic in the field of soil ecosystem. For a better understanding of the relationship between microbial diversity and soil function, BIOLOG EcoPlate was used to study the metabolic function variance of rhizosphere soil microbes [19]. It is known that BIOLOG is an effective method based on carbon substrate utilization by microbial communities to analyze data via multivariate statistics, including principal component analysis (PCA) and the dynamics of the microbial community, as it is revealed by BIOLOG metabolic variance [20].

Recently, a variety of culture-independent approaches—including random amplified polymorphic DNA, PCR-DGGE, and BIOLOG—have been applied for the investigation of bacterial diversity and metabolic function diversity of *P. ginseng* soil [4,21,22]. However, only a few trials have reported on the microbial community diversity of *P. ginseng* soil among different cultivation ages using both PCR-DGGE and BIOLOG. Therefore, it is of great interest to evaluate the influence of *P. ginseng* cultivation ages and modes on the bacterial and fungal genetic diversity and metabolic functional diversity with the combination of PCR-DGGE and BIOLOG, respectively. Findings from the present studies will help to elucidate variations in soil microbial community and to elucidate the status of microflora variation underlying the mechanisms of continuous *P. ginseng* farming, although further studies are required prior to practical application.

2. Materials and methods

2.1. Soil sampling and DNA extraction

Two fields—Yushu Village (Y; 42°32′N, 127°08′E, 537 m) and Funan Village (F or F′; 42°08′N, 127°32′E, 781 m), located in Jilin province, China—were selected for the experiments. A bioassay test was carried out, which comprised five cultivation ages—three different direct-seeding ages [direct seeding for 1 yr (R1), direct seeding for 2 yr (R2) and direct seeding for 4 yr (R4)] from Y, and two different transplanted ages [2-yr-old *P. ginseng* transplanted to another field for 2 yr (R2 + 2) and 3-yr-old *P. ginseng* transplanted to another field for 3 yr (R3 + 3)]; in addition, three cultivation modes—direct seeding mode, transplanted mode, and fallow soil of *P. ginseng* abandoned for a long period [fallow soil of *P. ginseng* abandoned in 2007 (RL-07) and in 2003 (RL-03) from F′ soil]—were compared with each other. Soil samples were collected from the rhizosphere soil of *P. ginseng* in growth stages (May 2014), and the forest soil of uncultivated *P. ginseng* (R0) was used as the control soil (Table 1).

At the beginning of the growing season, these fields were administered following good agricultural practice. Soil samples were collected from five replicate plots randomly distributed over the fields. The entire root system along with rhizosphere soil was collected by digging at a depth of 20 cm from five healthy plants, and sampling was performed as described previously [21,22]. Soil samples of different cultivation ages and modes of *P. ginseng* were used directly for DNA extraction using the UltraClean Soil DNA Isolation Kit (Mo Bio Laboratories Inc., Bohemia, NY, USA) according to the manufacturer's instructions [23].

2.2. PCR amplification and electrophoresis

The universal DNA Purification Kit [DP214, TIANGEN Biotech (Beijing) Co., Ltd., Beijing, China] was used for the PCR Clean-Up. On the basis of DNA extracted from *P. ginseng* soil, the variable region V3 of the 16S rDNA was amplified using primers GC-338F and 518R, designed to be specific for most bacteria for the analysis of bacterial diversity [24]. For the analysis of fungal diversity, PCR amplification of the 18S rDNA gene was performed using the fungal universal primers NS1 and GC-Fung [25].

2.3. DGGE community fingerprints, DNA sequences, and phylogenetic analysis

The PCR products were analyzed with DGGE using a BioRad DCode Universal Mutation Detection System (Bio-Rad, Richmond, CA, USA). Samples were subjected to 8% (w/v) polyacrylamide gels in 1× Tris-acetate-EDTA solution. Optimal separation was achieved with a 35–55% urea-formamide denaturing gradient for the bacterial community and 15–35% for the fungal community [100% denaturant corresponds to 7M urea and 40% (v/v) formamide]. Bacterial gel runs were performed for 4 h, whereas fungal gel runs

Table 1
Soil samples used for diversity analysis and main physicochemical characteristics ($\bar{x} + s$, $n = 3$)

Soil samples	Age (y)	Growth model[1)]	pH	Organic matter (g/kg)[2)]
R0 (control)	0	Forest soil uncultivated *Panax ginseng*	6.47 ± 0.04a	28.71 ± 1.99[a]
R1	1	1	5.74 ± 0.05b	27.48 ± 1.23[a,b]
R2	2	2	5.53 ± 0.04c	24.42 ± 1.39[b]
R4	4	4	5.44 ± 0.03c	14.39 ± 1.24[c]
R2 + 2	4	2 + 2	5.42 ± 0.02c	13.75 ± 0.78[c]
R3 + 3	6	3 + 3	5.21 ± 0.04c	10.96 ± 0.76[d]
RL-07	/	Fallow soil abandoned for 6 y	5.49 ± 0.03bc	6.37 ± 0.38[e]
RL-03	/	Fallow soil abandoned for 10 y	5.62 ± 0.04b	25.89 ± 0.79[b]

[1)] In the growth model column, $a + b$ means *Panax ginseng* growing at one place for a yr, then transplanted to another place and growing for b yr

[2)] The letters indicate the tested with Shortest Significant ranges (SSR) at P = 0.05 of different treatments. Different letters denote a significant difference at $p < 0.05$ level