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Comparison on the structure and function of the rhizosphere microbial community between healthy and root-rot *Panax notoginseng*

Zhaoxiang Wu[a,b], Zhipeng Hao[a], Yuqing Sun[a,b], Lanping Guo[c], Luqi Huang[c], Yan Zeng[d], Yong Wang[e], Li Yang[e], Baodong Chen[a,*]

[a] State Key Laboratory of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] State Key Laboratory of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China
[d] China National Group Corporation of Traditional and Herbal Medicine, Beijing, 100097, China
[e] Wenshan Sanqi Institute of Wenshan University, Wenshan, Yunnan 663000, China

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ABSTRACT

Root-rot disease, the main catastrophic disease of *Panax notoginseng* (Burk.) F.H. Chen, causes yield reduction and serious economic losses. However, knowledge of the relationship between rhizosphere microbial community and root-rot disease is limited. Therefore, the objective of this study was to evaluate the changes in the rhizosphere microbial community following the occurrence of *P. notoginseng* root-rot disease using three different techniques: phospholipid fatty acid (PLFA) profiling, 16S rRNA gene sequencing and functional-inference-based approaches (PICRUSt: phylogenetic investigation of communities by reconstruction of unobserved states). With paired comparison design, we studied the rhizosphere microbial community structure of healthy and root-rot *P. notoginseng* plants in Wenshan region of Yunnan province, southwest China. Microbial PLFA profiles showed that the abundance of total microbes, bacteria, fungi and the proportion of gram-negative bacteria in the rhizosphere soil of diseased plants were significantly higher than those of healthy plants. Furthermore, the proportion of gram-positive bacterial, actinomycetal and arbuscular mycorrhizal fungal PLFAs were significantly lower in the rhizosphere soil of diseased plants, and Proteobacteria was identified to be the marker phylum in rhizosphere soil of diseased *P. notoginseng*. By PICRUSt analysis, bacterial community function was predicted based on 16S rRNA sequencing. It was found that the potential function of microbial community from rhizosphere soil of diseased *P. notoginseng* differed significantly from that of healthy plants. Moreover, soil variables including soil texture and soil organic carbon significantly correlated with PLFA profiles, and were identified as important abiotic factors in shaping microbial communities.

1. Introduction

The health status of plant roots is the result of complex interactions between the plant, soil physico-chemical properties, local climate and soil microorganisms (Xu et al., 2012). Plants facilitate specific taxonomic or functional groups of microorganisms in the rhizosphere soil by producing exudates with diverse nutrients and secondary metabolites (Dennis et al., 2010). On the other side, soil microbes play key roles in soil biogeochemical processes including elemental cycling and energy flow. Microbial diversity and dynamics in soil largely determine soil health and are believed to be highly relevant to the outbreak or suppression of soil-borne plant diseases (Garbeva et al., 2004).

Panax notoginseng (Burk.) F.H. Chen, a well-known traditional Chinese medicine, usually called Sanqi or Tianqi in China, mainly distributed in Wenshan County, Yunnan province, southwest China. This medicine is effective for treatment of cardiovascular diseases, inflammation, different body pains, trauma, and internal and external bleeding caused by injury (Sun et al., 2005). *P. notoginseng* is usually cultivated in fields covered with adumbral net or branches with residual leaves of *Cunninghamia lanceolata* (Lamb.) Hook as it is sensitive to sunlight. High planting density, over fertilization, and growth condition that are damp and warm with reduced sunlight and air flow all provide a favorable environment for plant pathogens, especially root-rot disease,

* Corresponding author.
E-mail address: bdchen@rcees.ac.cn (B. Chen).

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which would consequently lead to crop yield and quality reduction.

The root-rot disease of *P. notoginseng* was found to be mainly associated with fungal pathogens such as *Alternaria panax*, *Alternaria tenuis*, *Cylindrocarpon destructans*, *Cylindrocarpon didymum*, *Fusarisum solani*, *Fusarisum oxysporum*, *Phytophthora cactorum*, *Phoma herbarum* and *Rhizoctonia solani*. It can also be caused by bacterial pathogens including *Pseudomonas* sp. and *Ralstonia* sp., and parasitic nematodes including *Ditylenchus* sp., *Rhabditis elegans* and *Meloidogyne* spp. (Miao et al., 2006). Previous studies have focused on single host/pathogen interactions, an unlikely scenario under natural conditions (Davies and Pedersen, 2008). One or several pathogen species mentioned above have shown an increase in the rhizosphere soil of root-rot *P. notoginseng*, but changes in the general rhizosphere microbial communities of diseased plants have not been studied. It is fundamentally important to compare the microbial community structure and function in the rhizosphere soil of healthy and diseased *P. notoginseng* to identify the microbial groups potentially relevant to the disease incidence.

Current available strategies to control root-rot disease of *P. notoginseng* mainly rely on chemical control and crop rotation (Yun et al., 2006). In recent years, due to the strict regulation of agrochemicals, chemical control has gradually become unviable. Biological control, as an environmentally friendly countermeasure, has been considered as a potential alternative. Endophytic microbes isolated from *P. notoginseng* were demonstrated to be effective in control of root-rot disease (Ma et al., 2013). However, in practice disease control appears to be more difficult, as it could be caused by the various pathogens mentioned above individually or in a complex. Integrated countermeasures should be developed to contend with multi-pathogens, while understanding the relationships between microbial community structure and environmental variables might provide some theoretical supports (Bulluck and Ristaino, 2002). Microbial communities have been shown to be impacted by abiotic factors, such as soil type and texture (Ulrich and Becker, 2006), pH (Lozupone and Knight, 2007), and carbon availability (Drenovsky et al., 2004), and also by biotic factors, such as root exudates (Dennis et al., 2010). Understanding how environmental changes affect soil microbial communities will help to predict soil health status and improve sustainable crop cultivation practices.

In the present study, phospholipid fatty acids (PLFAs) profiling and high-throughput sequencing were employed to analyze the composition of microbial community in the rhizosphere soil of both healthy and diseased *P. notoginseng*. Moreover, the bacterial community function was further predicted via PICRUSt based on 16 S rRNA sequencing. The objective of this study was therefore to characterize the structure and function of rhizosphere microbial community of healthy and root-rot diseased *P. notoginseng*, and to illustrate the effects of environmental factors on rhizosphere microbial community.

2. Materials and methods

2.1. Sampling site and experimental design

The research area was located in Wenshan region of Yunnan Province (103°35′–106°12′E, 22°40′–22°48′N), China, which produces the majority of the *P. notoginseng*. The area ranges from 1000 m to 1800 m in elevation. The mean annual temperature is 12.0–23.1 °C and mean annual precipitation is about 779 mm. The soil is Ferralic Cambisol (Soil Survey Staff, 2010) with a typical soil depth of 60–120 cm and pH ranging from 4.48 to 8.03, that is originally derived from Quaternary red claylimestone.

We adopted a paired comparison design to compare the rhizosphere microbial community between healthy and root-rot *P. notoginseng*. Nineteen *P. notoginseng* plantations were selected of which incidence of root-rot disease ranged from 5% to 70% that was calculated based on observation of typical root-rot symptoms (Xu et al., 2012). Each plantation was greater than 2 acres, and *P. notoginseng* plants had been grown for 2–4 years. Prior to this, wheat, maize or rice was cultivated in these fields.

2.2. Sampling and bioassay

The healthy and root-rot *P. notoginseng* in field was of great difference in appearance: chlorosis was obvious on diseased plants while the healthy plants grown well (Fig. S1). The healthy plants exhibited normal growth with no symptom of necrosis on root, however the diseased plants had wilted and etiolated leaves with clear symptoms on roots. We investigated 19 plantations, one composite sample was collected for both healthy (Hea) and diseased (Dis) plants, and two samples from each plantation, which resulted in 38 composite samples in total. Each composite sample was a mixture of 3–5 typical healthy or diseased plants and rhizosphere soil. The roots were carefully shaken to remove the loosely adhering soil, and the remaining attached soil was carefully collected by using sterile brushes and considered as rhizosphere soil (Riley and Barber, 1969; Courchesne and Gobran, 1997). All the soil samples and plant materials were kept on ice and transported to the laboratory within 6 h.

Soil samples were thoroughly mixed and passed through a 2 mm mesh to remove plant debris and stones. A portion of soil sample was freeze-dried and then kept at −80 °C for subsequent PLFA analysis and molecular analysis. Subsamples were air-dried for analysis of the basic soil chemo-physical properties. The fine roots were collected for determination of arbuscular mycorrhizal (AM) colonization after the plant materials had been rinsed with tap water and the remaining materials were dried at 60 °C for biomass assay. To visualize the AM fungal colonization, fresh roots were cleared by boiling in 10% KOH for 30 min, rinsed three times with tap water and stained by boiling in a 0.05% trypan blue solution for 30 min. Thirty randomly selected 1-cm root segments were examined using microscope at ×200 magnification according to the method described by Phillips and Hayman (1970).

2.3. Soil physico-chemical analysis

Soil pH was determined in a 1: 2.5 soil-water (w/v) slurry using a glass electrode pH meter (FE20-Five Easy Plus™, Switzerland). With a laser diffraction technique, the size distribution of soil particles were measured using a Long bench Mastersizer 2000 (Malvern Instruments, Malvern, England) as described by Wang et al. (2010), and according to the US soil classification system, the soil particle-size was fractionated into clay (0–2 μm), silt (2–50 μm) and sand (50–2000 μm). Soil particle size distribution (PSD) was characterized by the volume fractal dimension value (D value), which was calculated as description by Tyler and Wheatcraft (1992). Soil organic carbon (SOC) was determined by using the $K_2Cr_2O_7$ oxidation reduction titration method (Yuan et al., 2011). Soil inorganic nitrogen was measured using the boric acid absorption-hydrochloric acid titration method (Cornfield, 1960) and Olsen-P by Mo-Sb colorimetry method (Gyaneshwar et al., 2003). Soil available potassium was determined using flame spectrophotometers after soil samples were extracted with ammonium acetate as described by Portch and Hunter (2002). Total N, total S and total C of soil were determined by direct combustion using an element analyzer (Vario EL III, Germany), and C: N was calculated based on total C and total N (Hu et al., 2014).