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Research article

# Diversity, distribution, and antagonistic activities of rhizobacteria of *Panax notoginseng*

Ze-Yan Fan [1,2,4], Cui-Ping Miao [2,4], Xin-Guo Qiao [2], You-Kun Zheng [2], Hua-Hong Chen [3], You-Wei Chen [2], Li-Hua Xu [2], Li-Xing Zhao [2,*], Hui-Lin Guan [1,**]

[1] *School of Energy and Environment Science, Yunnan Normal University, Kunming, PR China*
[2] *Key Laboratory of Microbial Diversity in Southwest China of Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, PR China*
[3] *Department of Chemistry and Life Science, Chuxiong Normal University, Chuxiong, PR China*

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ABSTRACT

*Background:* Rhizobacteria play an important role in plant defense and could be promising sources of biocontrol agents. This study aimed to screen antagonistic bacteria and develop a biocontrol system for root rot complex of *Panax notoginseng*.
*Methods:* Pure-culture methods were used to isolate bacteria from the rhizosphere soil of notoginseng plants. The identification of isolates was based on the analysis of 16S ribosomal RNA (rRNA) sequences.
*Results:* A total of 279 bacteria were obtained from rhizosphere soils of healthy and root-rot notoginseng plants, and uncultivated soil. Among all the isolates, 88 showed antagonistic activity to at least one of three phytopathogenic fungi, *Fusarium oxysporum*, *Fusarium solani*, and *Phoma herbarum* mainly causing root rot disease of *P. notoginseng*. Based on the 16S rRNA sequencing, the antagonistic bacteria were characterized into four clusters, *Firmicutes*, *Proteobacteria*, *Actinobacteria,* and *Bacteroidetesi*. The genus *Bacillus* was the most frequently isolated, and *Bacillus siamensis* (Hs02), *Bacillus atrophaeus* (Hs09) showed strong antagonistic activity to the three pathogens. The distribution pattern differed in soil types, genera *Achromobacter*, *Acidovorax*, *Brevibacterium*, *Brevundimonas*, *Flavimonas,* and *Streptomyces* were only found in rhizosphere of healthy plants, while *Delftia*, *Leclercia*, *Brevibacillus*, *Microbacterium*, *Pantoea*, *Rhizobium,* and *Stenotrophomonas* only exist in soil of diseased plant, and *Acinetobacter* only exist in uncultivated soil.
*Conclusion:* The results suggest that diverse bacteria exist in the *P. notoginseng* rhizosphere soil, with differences in community in the same field, and antagonistic isolates may be good potential biological control agent for the notoginseng root-rot diseases caused by *F. oxysporum*, *Fusarium solani,* and *Panax herbarum*.


## 1. Introduction

*Panax notoginseng* F. H. Chen, known as Sanqi or Tianqi in Chinese, is a well-known traditional Chinese medicine [1], widely used for promotion of blood circulation, removal of blood stasis, induction of blood clotting, relief of swelling, alleviation of pain, and cure of coronary heart disease and cardiovascular disease [2]. Roots of *P. notoginseng* have been used as a variety of raw materials in Chinese medicinal products in China [3]. It has been mainly cultivated for 400 years in the Southwest regions of China, especially in Wenshan, Yunnan Province [4].

* Corresponding author. Li-Xing Zhao, Key Laboratory of Microbial Diversity in Southwest China of Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China.
** Corresponding author. Hui-Lin Guan, School of Energy and Environment Science, Yunnan Normal University, Kunming 650092, PR China.
*E-mail addresses:* zlx70@163.com (L.-X. Zhao), ghl0871@163.com (H.-L. Guan).
[4] These two authors contributed equally to this work.

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*P. notoginseng* should be grown in the field for at least 3 y to obtain high-quality raw roots [5]. However, the long period planting conditions make *P. notoginseng* vulnerable to attacks by many soil-borne pathogens including fungi, bacteria, and nematodes [5–14]. Soil-borne pathogens of *P. notoginseng* have been reported by fungi including *Fusarium oxysporum*, *Fusarium solani*, *Phoma herbarum*, *Alternaria tenuis*, *Alternaria panax*, *Cylindrocarpon destructans*, *Cylindrocarpon didynum*, *Phytophthora cactorum*, *Rhizoctonia solani*, and by bacterial pathogens including *Pseudomonas* sp., *Ralstonia* sp., and by parasitic nematodes, such as *Ditylenchus* sp., *Rhabditis elegans,* and *Meloidogyne* spp. [15,16]. In this case, the control of soil-borne diseases mainly relies on chemical pesticides, fungicides, and crop rotation. Chemical pesticides and fungicides are less effective on the soil-borne diseases, and lead to reduction of *P. notoginseng* quality. Meanwhile, pesticides may be toxic to crops, humans, animals [17,18]. However, a 15–20 y replanting interval leads to the lack of appropriate fields, resulting in searching for a new field or/and transferring to a less appropriate field to grow *P. notoginseng*.

It is obvious that pesticides and less appropriate cultivation soil are not suitable to control the qualities of *P. notoginseng* required by the good agriculture practice (GAP). Friendly approaches are urgently needed to effectively manage or solve the questions. Biological control, a bioeffector method with other living organisms to control pests (insects, mites, weeds, and plant diseases) [19], has been considered as effective approaches. Soil bacteria, especially rhizospheric ones with antagonistic properties, demonstrate biological control effectiveness to some plant diseases, and are the most potential for development of biological control agents (BCAs) [20–29]. However, little is known about the bacterial diversity, distribution, and ecological effects in the cultivation soil of *P. notoginseng*. In this study, we developed the investigation of rhizobacteria of 3-y-old *P. notoginseng* from Wenshan, Yunnan Province, by culture-dependent methods. The bacterial isolates were also challenged by three pathogens, *F. oxysporum*, *F. solani*, and *P. herbarum*, which are associated with the root rot disease of *P. notoginseng*.

## 2. Materials and methods

### 2.1. Soil sample collection and isolation of soil bacteria

Soil samples were collected from a 3-y-old *P. notoginseng* plantation in Wenshan, Yunnan Province, in July 2014. Ten healthy and 10 root-rot notoginseng plants were uprooted. Soil was collected around 3 cm from the main roots, and rhizosphere soil was gently stripped from the roots. Root-adjacent soil and rhizospheric soil were mixed together, recorded as healthy plant soil and diseased plant soil, respectively. Uncultivated soil sample was obtained without planting notoginseng at the same field. All the soil samples were placed into sterile plastic bags, transferred to the laboratory in 24 h, and kept at 4°C before treatment.

Bacterial isolation were developed using serial dilution spread plate method. Ten grams of soil was mixed with 90 mL of sterile phosphate buffered saline (PBS, pH 7.4) and stirred for 30 min at 200 rpm with a magnetic stirrer. The soil suspension was left to stand for 10 min at room temperature to allow settling of large particles, tenfold serial diluted in PBS (from $10^{-2}$ to $10^{-5}$). Then, 80 μL of the first to fourth and fifth diluents were transferred to petri dishes with LB agar medium (10.0 g peptone, 5.0 g yeast extract, 10.0 g NaCl, and 13.0 g agar, 1.0 L distilled water, pH 7.2) and nutrition agar (NA) medium (3.0 g beef extract, 5.0 g peptone, 5.0 g NaCl, 13.0 g agar, 1.0 L distilled water, pH 7.0). The plates were incubation at 28°C, and bacterial colonies were selected and purified according to their morphological characteristics.

### 2.2. Screening of antagonistic bacteria against fungal pathogens

Three fungal pathogens *F. oxysporum*, *F. solani*, and *P. herbarum* were isolated from the rotten root of *P. notoginseng*, and their pathogenicity was verified [15,16]. The target fungi were cultured on potato dextrose agar (PDA) medium (200.0 g fresh potato, 20.0 g starch, 13.0 g agar, 1.0 L distilled water, pH not adjusted). The antagonism of all bacterial isolates was checked with respect to the ability to suppress fungal growth. Antifungal bioassay was performed with the dual culture and agar well diffusion plate on PDA.

In dual culture tests, a 5-mm mycelial disk of pathogenic fungus, collected from the edge of actively growing colonies, was placed into the center of plates containing fresh PDA. Bacterial isolates were grown around the target fungus with a distance of 3.0 cm (Fig. 1A, 1B). The dual culture plates were incubation at 28°C, and checked every 12 h after inoculation. All treatments were tested in duplicate.

In agar well plate tests, a 200-μL fresh culture of pathogenic fungus with concentration of $10^8$ spores/mL was mixed with 250 mL PDA and evenly distributed into 10 petri dishes (90 mm). On each plate, four wells of 5 mm in diameter were made (Fig. 1C). Bacterial isolates were cultured in nutrient broth medium at 28°C, 135 rpm for 72 h. The bacterial suspension was adjusted to the final cell concentration of $10^7$ cfu/mL with nutrient broth medium. Next, 200 μL of suspension was added to each well, and the same volume of nutrient broth was used as control. All treatments were tested in duplicate.

### 2.3. Phylogenetic analysis

The genomic DNA of bacteria was extracted using a bacterial genomic DNA extraction kit (BioTeke Corporation, China, Cat#:

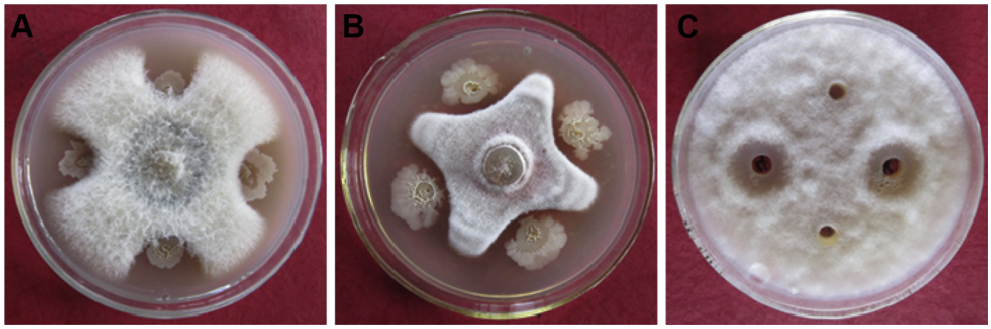


**Fig. 1.** Antagonistic assay of bacterial isolates (A) *Fusarium solani*, (B) *Phoma herbarum*, and (C) *Fusarium oxysporum*.