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Research article

Endophytic fungi harbored in *Panax notoginseng*: diversity and potential as biological control agents against host plant pathogens of root-rot disease

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ABSTRACT

Background: Endophytic fungi play an important role in balancing the ecosystem and boosting host growth. In the present study, we investigated the endophytic fungal diversity of healthy *Panax notoginseng* and evaluated its potential antimicrobial activity against five major phytopathogens causing root-rot of *P. notoginseng*.

Methods: A culture-dependent technique, combining morphological and molecular methods, was used to analyze endophytic fungal diversity. A double-layer agar technique was used to challenge the phytopathogens of *P. notoginseng*.

Results: A total of 89 fungi were obtained from the roots, stems, leaves, and seeds of *P. notoginseng*, and 41 isolates representing different morphotypes were selected for taxonomic characterization. The fungal isolates belonged to Ascomycota (96.6%) and Zygomycota (3.4%). All isolates were classified to 23 genera and an unknown taxon belonging to Sordariomycetes. The number of isolates obtained from different tissues ranged from 12 to 42 for leaves and roots, respectively. The selected endophytic fungal isolates were challenged by the root-rot pathogens *Alternaria panax*, *Fusarium oxysporum*, *Fusarium solani*, *Phoma herbarum*, and *Mycocentrospora acerina*. Twenty-six of the 41 isolates (63.4%) exhibited activity against at least one of the pathogens tested.

Conclusion: Our results suggested that *P. notoginseng* harbors diversified endophytic fungi that would provide a basis for the identification of new bioactive compounds, and for effective biocontrol of notoginseng root rot.

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1. Introduction

Panax notoginseng (Burkill) F.H. Chen (Araliaceae) is a perennial herbaceous plant, cultivated mainly in Wenshan, Yunnan, China, and has been historically used as both a medicinal herb and food. Rhizome and roots of *P. notoginseng* are officially recorded as notoginseng in the Chinese Pharmacopoeia [1]. About 61 Chinese patent medicines contain notoginseng, including Yunnan Bai Yao, a famous hemostatic proprietary herbal remedy. The secondary metabolites of this plant include saponins [2,3], flavones [2], amino

acids [4], and polysaccharides [5], with the most emphasis being on saponins. *P. notoginseng* saponins are considered as the major active ingredients in notoginseng. The saponins display multiple pharmacological effects, such as hemostatic [6–9], antioxidant [10,11], neuroprotective [12,13], antitumor [14,15], antidiabetic [16,17], and other activities, and have been extensively used as therapeutic agents in China. The pronounced efficacies of notoginseng saponins have led to the development of several Chinese patent medicines, such as Xuesaitong Capsules/Soft Capsules [18] and Xuesaitong Injection [19].

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In recent years, the demand for notoginseng has been increasing. Unfortunately, the yield and quality of notoginseng are severely limited by replanting obstacles [20], and a number of diseases caused by a plethora of phytopathogens [21]. Among the diseases, the root-rot disease complex is the most destructive one as it results in yield reduction, no harvest, or low content of active ingredients [22]. The reported fungal pathogens causing root rot include *Alternaria panax*, *Alternaria tenuissima*, *Cylindrocarpon destructans*, *Cylindrocarpon didymum*, *Rhizoctonia solani*, *Phytophthora cactorum*, *Phoma herbarum*, *Fusarium solani*, *Fusarium oxysporum* [21], and *Fusarium flocciferum* [23]. Although diverse chemical pesticides and some biocontrol methods are used, it is difficult to control the root-rot diseases because of the pathogenic complex. More comprehensive, practical, and ecological methods to eradicate the pathogenic diseases in *P. notoginseng* are urgently needed. Furthermore, specific and nonspecific fungi and bacteria associated with the plant have been found with little information about their ecological functions.

Microbial communities associated with plants play an important role in balancing the ecosystem and boosting host growth. Endophytic fungi, which live in healthy plant tissues for at least a part of their life cycle, without causing any noticeable symptoms of infection or disease, may benefit their host in different ways, such as producing bioactive secondary metabolites, promoting germination and shoot growth, inducing host plants to tolerate to biotic or abiotic stresses [24–27]. In a previous study, Ma et al [28] demonstrated high phylogenetic diversity and biocontrol

potential of bacterial endophytes of *P. notoginseng*. However, little is known about the fungal community harbored in *P. notoginseng*.

In this study, the diversity of endophytic fungi isolated from different tissues of healthy *P. notoginseng* was evaluated, and the isolates were screened for their potential antimicrobial activity against five major phytopathogens causing root rot of *P. notoginseng*. To the best of our knowledge, this is the first report of the biodiversity, phylogeny, and assessment of biocontrol potential of endophytic fungi harbored in *P. notoginseng*.

2. Materials and methods

2.1. Isolation of endophytic fungi

Three-year-old healthy *P. notoginseng* plant samples were collected in October 2013, from a plantation in Wenshan, Yunnan, Southwest China. The collected plants were excised into roots, stems, leaves, and seeds, put into plastic bags, transferred to the laboratory within 24 h, and stored at 4°C until the isolation procedure of endophytic fungi was carried out.

The surface sterilization and isolation of fungal endophytes were carried out by following the procedures described by Park et al [29]. The plant samples were washed thoroughly with running tap water to remove soil particles and rinsed six times with distilled water. The separated parts (roots, stems, leaves, and seeds; Fig. 1A) were immersed in 75% ethanol solution for 2–3 min, and subsequently transferred to 5.5% sodium hypochlorite solution for 1–2

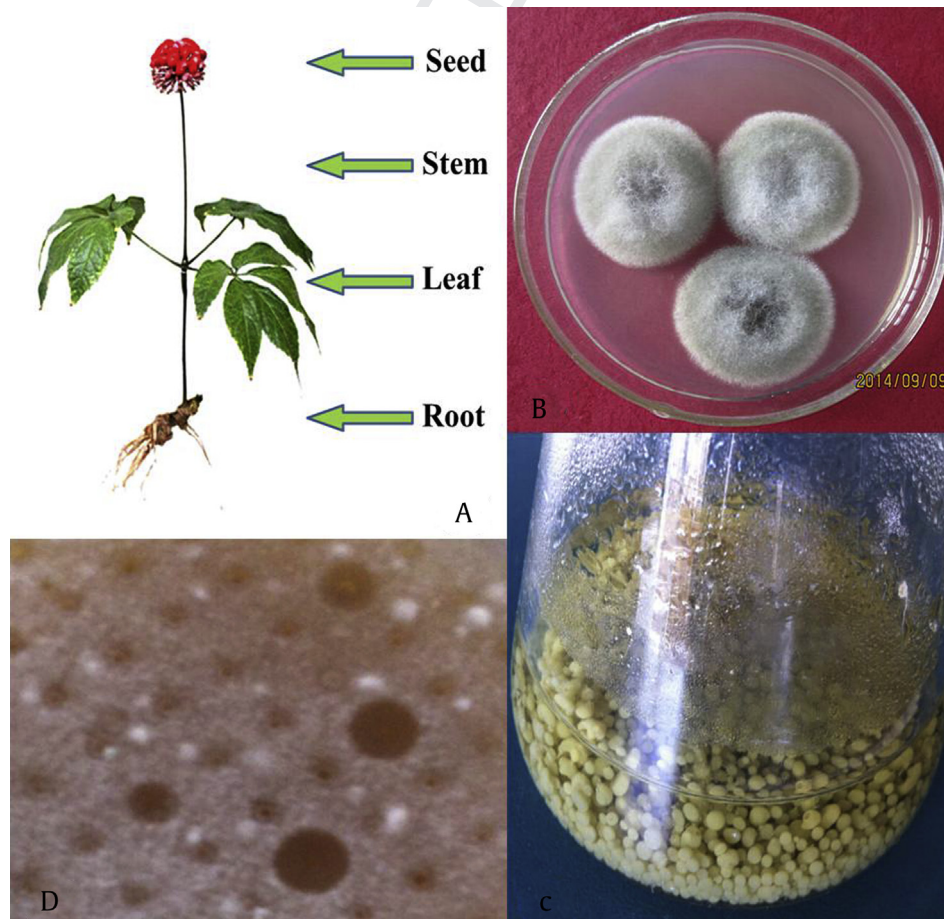


Fig. 1. *Panax notoginseng*, endophytic fungi and bioactivities. (A) Different parts of 3-year-old healthy plant of *P. notoginseng*; (B) isolation of endophytic fungi; (C) fermentation; (D) screening of antagonistic endophytic fungi against host phytopathogens of root-rot disease.

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