

Diversity of Endophytic Fungi Isolated from Korean Ginseng Leaves

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Abstract We investigated the diversity of the foliar endophytes of Korean ginseng. Endophytic fungi were isolated from healthy leaves of mountain-cultivated ginseng (MCG) and field-cultivated ginseng (FCG) at 4 sites in Chungbuk Province. A total of 24 species of fungal endophytes were identified using molecular approaches. Additionally, the diversity of these endophytic fungi was compared between MCG and FCG. The major isolated endophytes were *Edenia gomezpompae* and *Gibberella moniliformis* in the MCG and FCG samples, respectively. The results suggest that ginseng endophytes have different community structures in different environments, and this understanding may prove useful in ginseng cultivation.

Keywords *Edenia gomezpompae*, Endophytic fungi, *Gibberella moniliformis*, *Panax ginseng*

Endophytic fungi are defined as nonpathogenic fungi present in the tissues, leaves, branches, etc., of host plants [1]. Ascomycetes and Basidiomycetes comprise the majority of endophytic fungi [2], and these species are distributed ubiquitously in plants throughout the world. The relationships between host plants and endophytic fungi range from the positive (symbiosis) to the negative (parasitism) [3].

Panax ginseng C. A. Mey., also called Korean ginseng, is taxonomically classified in the family Araliaceae and the genus *Panax*. This species has a perennial life cycle and is a well-known medicinal plant throughout the world [4-6]. Korean ginseng prefers a shaded, humid area to a light, dry one and is distributed from 30° to 40° in the Northern Hemisphere; its distribution includes the Korean Peninsula, Northeast China, Japan, North America, etc. [7-9].

Korean ginseng has very low productivity compared to that of other crop plants, and the high susceptibility of both its shoots and roots to infection by various pathogens often disrupts its salability to markets [10]. Therefore, to

overcome these drawbacks, most ginseng fields are treated with agricultural chemicals. Recently, both domestic and foreign ginseng growers have begun to focus on sustainable production and clean technology, with a concurrent increase in the use of various helpful fungi for organic and clean farming [11, 12].

The organic cultivation of ginseng may be informed by the use endophytes and various microbes to control a late blight on tomato, an anthrax on cucumber, a powdery mildew on barley, etc. Previous studies have indicated the presence of symbiotic fungi living in ginseng roots [4, 12-14]; however, few reports have discussed endophytic fungi living in ginseng leaves. Therefore, this study investigates differences in the diversity and distribution pattern of endophytic fungi between the leaves of mountain-cultivated and field-cultivated ginseng (FCG).

MATERIALS AND METHODS

Samples. Ginseng leaves were collected from 4 sites (36°10'~37°07' N, 127°47'~128°11' E) in Chuncheongbuk-do during June 2010. Healthy leaves of FCG were collected from Jecheon-si and Yeongdong-gun, whereas healthy leaves of mountain-cultivated ginseng (MCG) were gathered from 2 sites in Cheongwon-gun. All plants had been cultivated for 5 yr, and 5 individual leaves were selected from 2 sections of each site. A total of 40 leaves were collected and transported to the laboratory using zipper bags, and the fungi were isolated from the leaves within 24 hr of collection.

Isolation of foliar endophytic fungi. The collected leaves were rinsed using tap water and washed again using a sonicator (JAC-2010; KODO, Hwaseong, Korea) to remove

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extraneous substances from the leaf surfaces. For surface sterilization, the leaves were cut into 1 × 1 cm sections, then submerged for 1 min in 96% ethyl alcohol, 3 min in 1% NaOCl, and 30 sec in 96% ethyl alcohol and rinsed in sterile water twice. Sections from each leaf were grown on 3 different media (0.5% malt extract agar, potato dextrose agar, water agar; petri dishes 87 × 15 mm) at 25°C for 4 wk in darkness to isolate the endophytic fungi [15].

Identification of endophytic fungi using a sequence-based approach. The isolated fungi were subcultured to obtain pure fungal strains, and these strains were used for DNA extraction following the protocol of the Plant DNA Isolation MiniKit (GeneAll Exgene Plant SV; GeneAll Biotechnology, Seoul, Korea). PCR was performed using the primers ITS1F and ITS4 to amplify specific fungal internal transcribed spacer regions [16]. The PCR conditions were as follow: 5 min at 94°C for pre-denaturation, then 30 sec at 94°C for denaturation, 1 min at 55°C for annealing, and 1 min at 72°C for elongation (1 cycle). The steps from denaturation to elongation were repeated for 30 cycles, followed by a final stabilization step of 5 min at 72°C for stabilization. The PCR products were examined using

electrophoresis on 1.5% agarose gels and stored at 4°C. Sequencing of the PCR products was conducted in Solgent (Daejeon, Korea), and the sequences were analyzed using BLAST (Basic Local Alignment Search Tool) on the NCBI website (<http://www.ncbi.nlm.nih.gov/>) to find the sequences with the greatest similarity to our sequences. The identified sequences were listed, and alignment was conducted to construct a phylogenetic tree by neighbor-joining, with a bootstrap value of 1,000, using MEGA 5.05 [17]. Finally, using the identified taxa, we compared the endophyte species diversity of the MCG and FCG samples using the Shannon diversity index (H') [18]. Fungal strains isolated from this study were deposited in Korea National University of Education Culture Collection and the sequences were deposited into GenBank under accession Nos. KJ957770~KJ957793.

RESULTS AND DISCUSSION

We isolated 53 endophytic fungal strains from the 40 ginseng leaves. These fungal strains were divided into 24 taxa using morphological and molecular methods (Table 1). These taxa were grouped into 4 classes, excepting the fungal strains of *Incertae sedis*: Sordariomycetes, Dothideomycetes,

Table 1. Molecular identification of endophytic fungi from leaves of *Panax ginseng* using internal transcribed spacer region

Strain (accession No.)	Results of BLAST search on NCBI			Relative abundance ^a	
	The closet Genbank taxa	Accession No.	Similarity (%)	FCG ^b	MCG ^c
11G001(KJ957770)	<i>Lecytophora hoffmannii</i>	AB231012.1	99	3.7	-
11G002 (KJ957771)	<i>Coniochaeta ligniaria</i>	JQ619824.1	93	3.7	-
11G003 (KJ957772)	<i>Lecytophora</i> sp.	JX243746.1	99	3.7	9.6
11G005 (KJ957773)	<i>Hypoxyton perforatum</i>	KC507200.1	99	3.7	-
11G006 (KJ957774)	<i>Hypoxyton ticinense</i>	JQ009317.1	95	3.7	-
11G007 (KJ957775)	<i>Coniochaeta</i> sp.	HQ657313.1	99	3.7	-
11G008 (KJ957776)	<i>Nemania</i> sp.	KC507255.1	100	3.7	-
11G009 (KJ957777)	<i>Loptospora rubella</i>	KC172076.1	99	3.7	-
11G013 (KJ957778)	<i>Lophiostoma</i> sp.	GU827618.1	99	3.7	-
11G014 (KJ957779)	<i>Cerraena consors</i>	FJ821528.1	100	3.7	-
11G017 (KJ957780)	<i>Colletotrichum</i> sp.	DQ286215.1	99	3.7	-
11G019 (KJ957781)	<i>Acremonium</i> sp.	HQ649795.1	99	-	4.7
11G020 (KJ957782)	<i>Hypoxyton notatum</i>	KF177678.1	90	-	4.7
11G026 (KJ957783)	<i>Colletotrichum panacicola</i>	GU935869.1	99	3.7	19.2
11G029 (KJ957784)	<i>Stemphylium solani</i>	JQ936290.1	99	-	4.7
11G033 (KJ957785)	<i>Cladosporium</i> sp.	AJ971408.1	99	-	4.7
11G037 (KJ957786)	<i>Gibberella moniliformis</i>	JX914478.1	99	-	23.8
11G039 (KJ957787)	<i>Aureobasidium</i> sp.	KF367567.1	99	3.7	4.7
11G043 (KJ957788)	<i>Hansfordia</i> sp.	GQ906965.1	99	3.7	-
11G044 (KJ957789)	<i>Daldinia childiae</i>	AM292044.1	99	11.1	-
11G045 (KJ957790)	<i>Aspergillus</i> sp.	JQ717330.1	99	3.7	9.6
11G047 (KJ957791)	<i>Colletotrichum gloeosporioides</i>	JF441191.1	99	7.4	9.6
11G048 (KJ957792)	<i>Edenia gomezpompae</i>	JX868710.1	99	22.3	-
11G049 (KJ957793)	<i>Alternaria alternata</i>	JF440581.1	99	3.7	4.7
Total No. of isolates				27	21
Shannon diversity index (H')				2.72	1.22
Species richness				19	11

^aRelative abundance: the percentages of the number of isolates in the study sites of the total numbers of isolates.

^bCultivated ginsengs in the field.

^cCultivated ginsengs in the mountain.

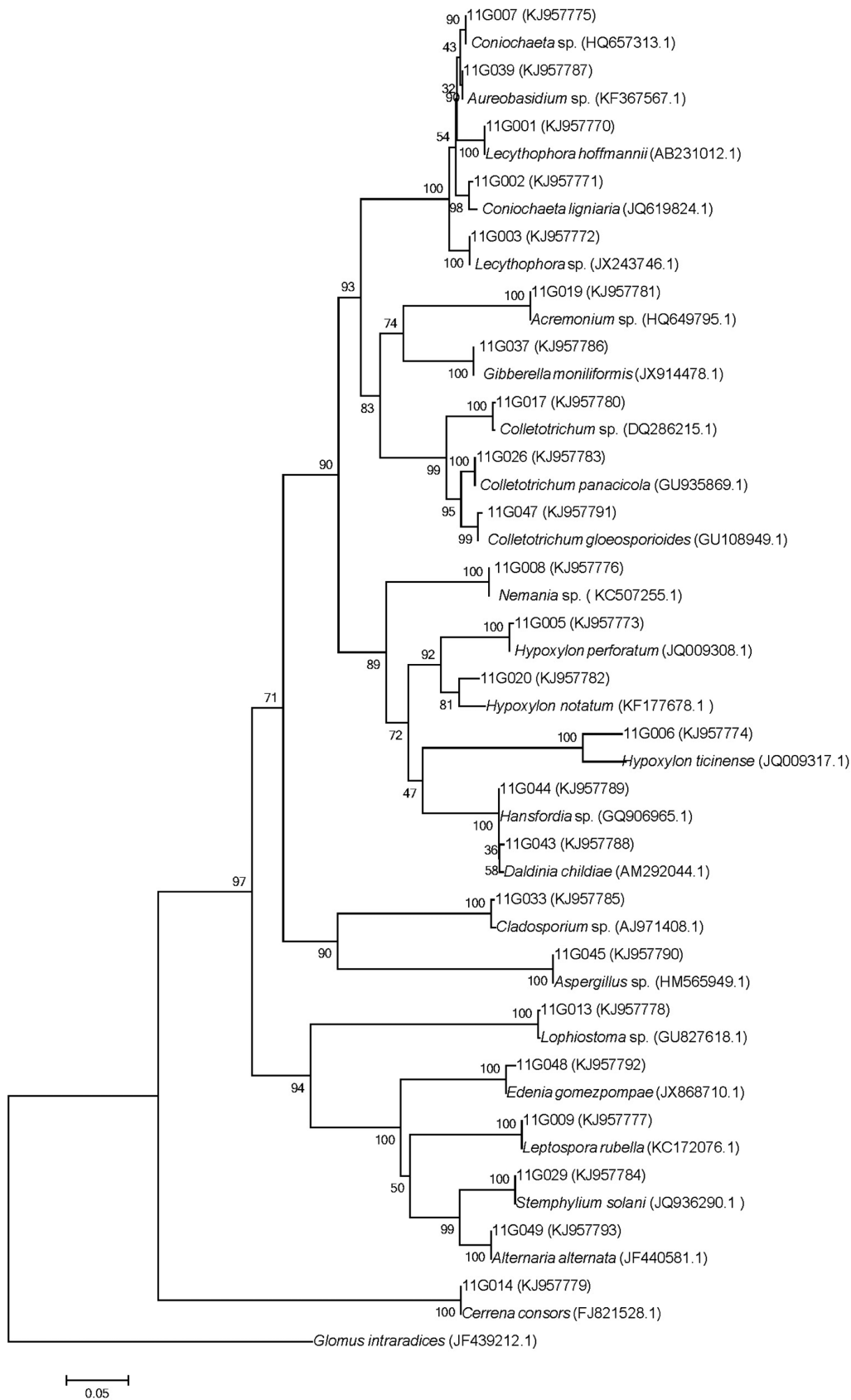


Fig. 1. Phylogenetic tree of endophytic fungi from *Panax ginseng* in field and mountain cultivated land. Internal transcribed spacer and 5.8S rDNA region were used for the sequence analysis to confirm the topological appropriation of the fungal isolates. *Glomus intraradices* was used as an out-group and bootstrap values are shown at the branches (1,000 replicates).

Agaricomycetes, and Eurotiomycetes (Fig. 1).

A total of 11 endophytes were found in MCG, whereas 19 endophytes were found in FCG. In MCG, *Gibberella moniliformis* and *Colletotrichum panicola* were the dominant species, whereas *Edenia gomezpompae* and *Daldinia childiae* were the dominant species in FCG. Only 5 taxa were found in both MCG and FCG: *Aspergillus* sp., *Alternaria alternata*, *Lecythophora* sp., *Colletotrichum gloeosporioides*, and *C. panicola*. The species diversity of endophytes observed in FCG ($H' = 2.72$) was twice that in MCG ($H' = 1.22$). Generally, the species diversity of endophytes is related to host plant, season, humidity, etc. [17, 19]. In this study, cultivation method was also identified as an element influencing endophytic species diversity. In the case of FCG, agricultural chemicals and fertilizers were employed to increase ginseng quality and harvest yield, whereas MCG was grown in a natural environment without the use of agricultural chemicals; cultivation methods differ widely between FCG and MCG [20]. Host plants grown in natural conditions are generally predicted to have higher endophytic species diversity because of the higher frequency of contact between fungi and host plants than in a normal cultivation environment, but the results of the present study suggest an opposite outcome. Many factors may explain this situation, but the health of the host plants and the life strategies of endophytic fungi (parasitic to symbiotic) are thought to be major components. The health of host plants affects the implantation and infiltration of endophytes [21]. In this context, the traditional large-scale, single-crop cultivation of ginseng involves repeated damage to the plants through the application of agricultural chemicals and fertilizers, allowing various fungal species to colonize ginseng more easily. While endophytic fungi may have a higher rate of contact with ginseng plants under MCG, these plants also have a higher resistance to the implantation of fungal species; therefore, endophytes have a higher frequency in FCG than in MCG. Additionally, pathogens may kill plants naturally under MCG but cannot kill them under FCG due to artificial maintenance of the crop using agricultural chemicals and fertilizers. Ginseng plants may then carry germs and spread dormant pathogens to healthy individuals. Secondary infection through the horizontal spread of endophytes may help explain the gap in diversity between MCG and FCG.

When comparing these results with those of other research, no endophytic taxa are common between ginseng leaves and roots, even at the genus level [22]. Three taxa at the genus level identified in this study were shared with the endophytic fungi community of *P. quinquefolium* L., which is cultivated in China: *Alternaria*, *Cladosporium*, and *Colletotrichum*. Most of the endophytes identified in that species, however, were different from those of the present study [23]. This result supports the leading hypothesis that endophyte communities are influenced by geological, climatic, and botanical factors [19, 24].

The identification of both *Gibberella moniliformis* in MCG and *Edenia gomezpompae* in FCG represents the first

detection of these species in ginseng leaves. *Gibberella moniliformis* has been previously reported as a harmful species to crops, but as an endophyte, it can stimulate the growth of host plants through symbiosis [25]. This endophyte species may promote the growth of MCG and help it compete against herbaceous plants. *Edenia gomezpompae* has been reported as an endophyte with a distribution primarily in warm and dry regions rather than temperate regions [26]. These species have previously been reported as having symbiotic connections with the Lamiaceae, Fabaceae, and Poaceae; we are the first to report the isolation of this species from the Araliaceae. In light of these reports, *Edenia gomezpompae* may be distributed on both monocotyledons and dicotyledons, as well as both herbaceous and woody host species, and the species was commonly isolated from the leaves of the host plants in this study [27]. In some conifers, however, such as *Pinus densiflora* Siebold et Zucc., *Pinus koraiensis* Siebold et Zucc., etc., *Edenia gomezpompae* has not been found; this situation represents an interesting avenue for future research.

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