Diversity of endophytic fungi from roots of *Panax ginseng* and their saponin yield capacities

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**Abstract**

Endophytes of medicinal plants have the capacity to synthesize same or similar active substances with their hosts. To investigate the diversity and capacity to produce saponins of endophytic fungi of *Panax ginseng*, thirty-eight strains were isolated. Polymerase chain reaction (PCR) and sequencing were used to identify the isolates, and saponins concentrations in the cultures were measured. Agar diffusion method was used to test antimicrobial activity. High-performance liquid chromatography (HPLC) was used to analyze ginsenosides produced by representative strains. *Nectria*, *Aspergillus*, *Fusarium*, *Verticillium*, *Engyodontium*, *Plectosphaerella*, *Penicillium*, *Cladosporium*, and Ascomycete species were isolated. Overall, 18.4% of the isolates belonged to *Nectria* (*Nectria haematococca*), 13.2% belonged to *Aspergillus*, and 10.5% belonged to *Penicillium*. The highest concentration of triterpenoid saponin was 0.181 mg/ml (Pg27), followed by 0.144 mg/ml (Pg30 and Pg42-1). According to the results of the phylogenetic results, these isolates were species of *Fusarium*, *Aspergillus* and *Verticillium*, respectively. The culture filtrate of Pg30 exhibited its antibacterial activity against *Staphylococcus aureus*. Pg 27 and Pg30 could excrete the substances to inhibit the growth of *Rhizoctonia solani*. Pg42-1 showed strong inhibition against *Klebsiella pneumoniae*. From HPLC results, the ginsenoside Rb2 was detected in both Pg27 and Pg30 cultures. The ginsenoside Rc was found in Pg42-1 cultures. In conclusion, thirty-eight endophytic fungal strains were isolated and Pg27 (*Fusarium* sp.) has a potential application value in saponins production.

**Keywords:** Diversity, Saponin, Endophytic fungi, *Panax ginseng*, Ginsenoside

**Introduction**

Ginseng is one of the most famous medicinal plants in the *Araliaceae*, which occupies an important position in traditional Chinese medicine in China. With the excessive and predatory exploitation, wild ginseng resources become scarce. Cultivated ginseng has gradually become the mainstream of the market. To develop the ginseng farming, deforestation reclaimed to new participants is necessary, because the humus in forest area is essential for ginseng cultivation. However, this deforestation has greatly broken the ecological balance and biodiversity in forest area. How to obtain medicinal ingredients from ginseng without damage to the environment has become a very important issue.

Endophytic fungi, which are fungi that colonize a plant without causing visible disease symptoms (Schulza and Boyle 2005), are common in plants (Lin et al. 2010; De Siqueira et al. 2011; Suto et al. 2002), and have been found to be ubiquitous within all examined plants (Sun et al. 2011; Tadych et al. 2012; Li et al. 2012). In addition, endophytic fungi have been isolated from different plant tissues, including flowers, seeds, roots, stems and leaves (Lupo et al. 2001; Bayman et al. 1997). Previous studies have found that some endophytic fungi have roles within the plant in relation to growth (Doty 2011), enhanced stress resistance (Ownley et al. 2010), degradation of pollutants (Sun et al. 2011), and the production of bioactive substances in the host (Guimarães et al. 2008).

In medicinal plants, some endophytic fungi have been found to produce secondary metabolites that have medicinal value. Indeed, since the discovery that the endophytic fungi isolated form *Taxus brevifolia*, *T. celebica*, *T. mairei*, *T. chinensis var. mairei*, and *T. wallachiana* produced the anti-cancer drug taxol, many researchers have studied the endophytic fungi of medicinal plants to identify potential sources of novel medicine (Lin et al. 2007; De Siqueira et al. 2011; Kumaran et al. 2010). Saponin is the main...
medicinal product of *Panax ginseng* and has multiple therapeutic values, including anti-tumor and anti-aging properties and blood vessels softening. Studying the saponin yield capacity of *Panax ginseng* endophytes could provide new sources for producing saponins and protect wild ginseng resources indirectly. Antimicrobial activity of endophytes is also one research direction. Endophytes and their metabolites are generally not harmful to their host. Therefore, endophytes which are resistant to pathogens may become the natural sources for pesticides (Yang et al. 2006).

Researches on ginseng endophytes mainly focused on the diversity and the biological activity of metabolites. Xu et al. isolated *Paecilomyces* sp. from the ginseng and studied its antifungal and antitumor properties. The results showed that the extracts derived from *Paecilomyces* sp. and ginseng samples contained the same compound falcarnicol, an atural pesticide and anti-cancer agent (Xu et al. 2009). Park et al. isolated 38 fungal isolates from *Panax ginseng* in Korea. They were classified into *Phoma radicina*, *Fusarium oxysporum*, *Setophoma terrestris* and *Ascomycota* sp. 2-RNK. The most dominant fungal endophyte was *P. radicina* in 3 cultivars (Park et al. 2012). In the present study, to select the endophytes with the capacity of producing saponins, we investigated the diversity of the endophytic fungi in the roots of *Panax ginseng* cultivated in the forest of Northeast China. The saponin concentrations of typical strains were measured. The antimicrobial activity of representative strains was tested and ginsenosides produced by typical strains were analyzed.

**Materials and methods**

**Sampling and isolation**

*Panax ginseng* (PG) specimens were respectively sampled from Fu-yuan City and Ji-an City (Jinlin, China) and had been grown for 15 years in the forest. The PG samples were immediately put into sterile plastic bags and stored at 4°C. The endophytes were isolated within 48 hours. Before disinfection, the plant samples were thoroughly washed under running tap water for 10 h. The PG root samples were surface-disinfected with 70% (v/v) ethanol for 1 min, 5% NaOCl 10 min, 70% (v/v) ethanol for 1 min and burning for 30 sec. The samples were subsequently rinsed with sterile water, and the outer tissue was removed with a sterile scalpel. Small pieces (0.5×0.5 cm) of PG were placed in Petri dishes containing malt extract agar (MEA, Difco, USA), Czapek agar (CZA, Difco, USA), or potato dextrose agar (PDA, Difco, USA), and incubated at 28°C for five days. Following the incubation, single colonies of distinctive morphotypes were separated on the basis of their morphological characteristics and appearance. The colonies were subsequently re-isolated by plating on PDA and incubated at 28°C for 24–48 h to obtain pure cultures. All of the isolates were vacuum freeze-dried and deposited in the College of Life Sciences, Northeast Forestry University.

**DNA extraction and PCR amplification of the 28S rRNA gene**

The genomic DNA was extracted using the EZNA Fungal DNA Mini Kit (OMEGA, USA) according to the manufacturer's instructions. The 50 μl PCR mixtures contained 15 ng of template DNA, 1× PCR buffer (Mg²⁺ free), 0.16 mM of each dNTP, 1.5 mM MgCl₂, 0.45 μM of each primer, and 1 U of Takara *rTaq* DNA polymerase (Takara, Japan). The primers for the amplification of the D1/D2 region of the fungal 28S rRNA gene were NL1 (5'-GCTATCAATAAAGGCGGAGGAAAG-3') and NL4 (5'-GCTCGGTCTTTCAAGACGG-3') (Redecke 2000). The thermocycler program consisted of initial an DNA denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 45 s, and elongation at 72°C for 1 min 30 s, and ending with a final elongation step at 72°C for 6 min (Yang et al. 2007).

The PCR amplification products were separated by electrophoresis through 1% (W/V) agarose gels and stained with ethidium bromide for visual examination. The PCR products were purified using the Agarose Gel DNA Extraction Kit (Takara, Japan) and sequenced at Sangon Biotech (Shanghai, China).

**Phylogenetic analysis and nucleotide sequence accession numbers**

The sequences generated in this study were compared with those in GenBank (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the sequences with a similarity ≥99% to the partial 28S rDNA regions (a. 600 bp) were considered to belong to identical genera. A neighbor-joining tree (Thompson et al. 1997) was constructed using MEGA 5.0 software (Tamura et al. 2011). The number of bootstrap replications was 1000. The sequences were deposited in GenBank under the accession numbers shown in Table 1.

**Determination of triterpenoid saponins**

Each isolate was cultured in 100 ml PDA liquid medium (250 ml flask), and stirred at 150rpm at 28°C for two weeks. After ultrasonication, the supernatant was separated from the cell debris by centrifugation at 4,000 × g for 20 min. A 20 ml aliquot of the supernatant was poured into a 50 ml centrifuge tube (Corning, USA), and 20 ml ethyl acetate was added into the same tube. After mixing, ultrasonication and incubation for 5 min, 5 ml of the supernatant was evaporated to dryness under a vacuum at 50°C. The residue was dissolved in 2 ml methanol. The methanol solutions were centrifuged at 4,000 × g for 10 min, and the supernatants were used for the subsequent analysis of the total saponins and ginsenosides.
| Strain ID | Accession No. | Closest species (Accession No.)                                      | Similarity (%) |
|----------|---------------|---------------------------------------------------------------------|----------------|
| Pg31     | JQ807916      | Verticillium sp. (AY312607)                                         | 99.5           |
|          |               | Engyodontium album (HM214541)                                       | 99.3           |
|          |               | Engyodontium album (DQ872372)                                       | 97.9           |
| Pg50-1   | JQ807940      | Fusarium sp. (AB294824)                                            | 100            |
| Pg14     | JQ807941      | Fusarium sp. (AB294823)                                            | 100            |
|          |               | Fusarium solani (AB363765)                                         | 100            |
| Pg33-2   | JQ807905      | Penicillium guttulatum (HQ646592)                                   | 100            |
| Pg33     | JQ807906      | Penicillium menonorum (HQ646591)                                    | 100            |
|          |               | Penicillium menonorum (HQ646590)                                    | 99.8           |
| Pg42-1   | JQ807917      | Verticillium sp. (HM057107)                                         | 100            |
|          |               | Verticillium psalliotae (AF500907)                                  | 100            |
|          |               | Verticillium psalliotae (AB378520)                                  | 99.6           |
| Pg44     | JQ807958      | Uncultured Ascomycota (HQ432963)                                    | 100            |
| Pg4-2    | JQ807959      | Plectosphaerella cucumerina (JF780520)                              | 100            |
| Pg64     | JQ807960      | Plectosphaerella cucumerina (HQ239034)                              | 100            |
| Pg4-1    | JQ807961      |                                                                   |                |
| Pg42     | JQ807911      | Aspergillus fumigatus (JQ268555)                                    | 100            |
| Pg41-2   | JQ807912      | Aspergillus fumigatus (JN938928)                                    | 100            |
|          |               | Aspergillus fumigatus (AB354184)                                    | 100            |
| Pg34     | JQ807970      | Fungal sp. (GU552503)                                              | 99.6           |
|          |               | Fusarium oxysporum (FJ614650)                                       | 99.6           |
|          |               | Fusarium sp. (AB373725)                                            | 99.6           |
| Pg50     | JQ807951      | Penicillium simplicissimum (HM469430)                               | 99.5           |
| Pg50-1   | JQ807940      | Penicillium sp. (HM469409)                                          | 99.5           |
|          |               | Penicillium brasiliannum (HM469396)                                 | 99.5           |
| Pg61     | JQ807988      | Uncultured Ascomycota (HQ433122)                                    | 95.6           |
|          |               | Uncultured Ascomycete (HQ432972)                                    | 95.5           |
|          |               | Uncultured Ascomycete (EU489938)                                   | 93.4           |
| Pg32     | JQ807987      | Penicillium guttulatum (HQ646592)                                   | 92.4           |
|          |               | Penicillium menonorum (HQ646591)                                    | 92.4           |
|          |               | Penicillium menonorum (HQ646590)                                    | 92.2           |
| Pg10     | JQ807978      | Uncultured Ascomycota (HQ433122)                                    | 99.6           |
| Pg5      | JQ807980      | Paraphoma chrysanthemica (GQ387582)                                 | 98.9           |
| Pg63     | JQ807979      | Paraphoma chrysanthemica (GQ387583)                                 | 98.9           |
| Pg12-1   | JQ807937      | Fusarium sp. (AB294826)                                            | 100            |
|          |               | Nechta haematococca (DQ119558)                                      | 99.8           |
|          |               | Nechta haematococca (HM042416)                                      | 99.8           |
| Pg36     | JQ807948      | Fungal sp. (GU552494)                                              | 100            |
|          |               | Neectoria radicicola (HM364304)                                     | 99.6           |
|          |               | Neectoria radicicola (U17415)                                       | 99.3           |
| Pg34     | JQ807970      | Cladosporium sp. (FJ790290)                                         | 100            |
|          |               | Cladosporium cladosporioides (AY213695)                             | 100            |
|          |               | Passalora fulva (AB100653)                                         | 100            |
The measurement of the total extracted saponins was based on a color reaction of the acid-hydrolysis products of the saponins (i.e. sapogenins) with vanillin. In total, 5 ml of the supernatant was added to a test tube and evaporated at 60°C in a water bath. The residue was dissolved in 0.2 ml 5% vanillin, mixed with 0.8 ml perchloric acid, incubated at 60°C in a water bath for 15 min and quickly cooled in ice water. The concentration of saponins (mg/ml) in the reaction sample was detected using a spectrophotometer at 560 nm against a calibration curve established with an oleanolic acid standard (National Institutes for Food and Drug Control, Beijing, China) (Liu et al. 2011).

**Antimicrobial activity of the representative strains**

The 14-day culture filtrates were assessed for antimicrobial activity by the agar diffusion method (Hormazabal and Piontelli 2009) against the test microorganism strains showed as Table 3. Three 6-mm wells were made in each disk. Culture filtrates (0.2 ml) was added in each well. Except for *Fusarium sporotrichioides* (isolated in our lab), the other strains were purchased from Agricultural Culture Collection of China (ACCC). As a reference, the Streptomycin Sulfate (5 mg/well), the Amoxicillin (5 mg/well) and the Itraconazole Hydrochloride (4.4 mg/well) were used as antibacterial standards. The activity of the extracts was estimated from growth inhibition (in mm).

**Ginsenosides analyses**

A 100 ml ethyl acetate was added the 100 ml liquid culture. After 30 min agitation at 160 rpm and ultrasonication at 50°C, the supernatant was separated from the cell debris by centrifugation at 4,000 × g for 30 min. After evaporation, the pellet was dissolved with a 5 ml methanol, then filtrated with SepPak C-18 Cartridge (Waters, USA). Standards were purchased from National Institutes for Food and Drug Control (Beijing, China). Acetonitrile (DIKMA, USA) and water were HPLC grade. HPLC analysis were performed using Separations Module (Model e2695, Waters, USA), photodiode Array Detector (Model 2998, Waters). Sample volume was 10 μl. The wavelength of the detector is 203 nm. Ginsenoside was analyzed using a XTerra® MS column C-18, 5 μm, 4.6 mm × 2.5 mm. The mobile phase consisted of a mixture, acetonitrile : water (0-40 min, 18:82–18:82, v/v; 40-50 min, 18:80–22:78 v/v; 50-70 min, 22:78–28:72 v/v; 70-100 min, 28:72–38:62 v/v; 100-110 min, 38:62–18:82 v/v). The flow was of 1.0 ml min⁻¹ and the sensitivity was 0.001 AUFS. The HPLC system was operated at room temperature (25 ± 1°C).

**Results**

**Similarity of the sequences**

Thirty-eight strains were identified on the basis of their morphological characteristics. The sequences were compared...
with those in the GenBank database, and the results are shown in Table 1.

**Phylogenetic analysis**

The phylogenetic tree built from the 28S rDNA sequences is shown in Figure 1. Nine fungal genera were identified: *Nectria, Aspergillus, Fusarium, Verticillium, Engyodontium, Plectosphaerella, Penicillium, Cladosporium, and Ascomyete*. The most representative genera were *Nectria, Aspergillus, and Penicillium*: 18.4% belonged to *Nectria* (*Nectria haematococca*), 13.2% belonged to *Aspergillus*, and 10.5% belonged to *Penicillium*.

**Analysis of triterpenoid saponins**

The concentration of triterpenoid saponins of typical isolates are showed in Table 2. The highest concentration of saponins was 0.181 mg/ml in Pg27, which was significantly higher than Pg30 and Pg42-1 (0.144 mg/ml) (P<0.05). According to the results of phylogenetic results, Pg27 was identified as a *Fusarium* sp., Pg30 was identified as an *Aspergillus* sp., and Pg42-1 was identified as a *Verticillium* sp. The saponin concentrations among the strains of the same genus were different significantly (P<0.05), for example Pg14 (0.023 mg/ml), Pg34 (0.133 mg/ml) and Pg12-1 (0.042 mg/ml); these isolates were also identified as *Fusarium* spp.

**Antimicrobial activity**

To test the Pg27, Pg30 and Pg42-1 potential use, the antimicrobial activity was analyzed. From Table 3, the culture filtrate of Pg30 exhibited its antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* ACCC10499. Pg27 and Pg30 could excrete the substances to inhibit the growth of *Rhizoctonia solani* ACCC36233, which was a pathogenic fungi of *Panax notoginseng*. The culture filtrate of Pg42-1 showed strong inhibition against *Klebsiella pneumoniae* ACCC10498. This result indicated that Pg42-1 might be a potential medical source.

**Ginsenosides analyses**

According the result of total saponins, Pg27, Pg30 and Pg42-1 produced higher concentrations of saponins. To further analyze the composition of saponins, the standards of eight ginsenosides were injected into HPLC. The spectrums showed as Figure 2. Rb2 was detected in both Pg27 and Pg30 cultures. The concentration of Pg30 was especially high. Rc was found in Pg42-1 cultures.

**Discussion**

Thirty-eight endophytic fungi were isolated, and they were classified into nine genera according to the morphological types and 28S rDNA sequencing results. Three isolates (Pg45, Pg47, and Pg60) were not identified because of lack of comparative sequences: their sequences were

![Figure 1 Phylogenetic tree based on 28S rDNA sequences using Neighbor-Joining method. Scale bar indicates 20% estimated sequence divergence.](image-url)
significantly similar to unknown fungal sequences in the GenBank database. *Nectria*, *Aspergillus* and *Penicillium* were the predominant genera. The host materials were all healthy in this study. Park et al. (2012) reported that *Phoma radicina*, *Fusarium oxysporum*, *Setophoma terrestris* and *Ascomycota* were the predominant endophytic fungi in Korean Ginseng, and Xing et al. (2010) reported that *Cladosporium* sp. was the dominant species in the root of *Panax quinquefolium*. These previous results were different from this present study, indicating the specificity of the endophytic fungi from different areas and plants.

*Nectria* was reported as endophytic fungi in European beech (Danti et al. 2002) and red alder (Dorworth et al. 1996) as the endophytic fungi. *Nectria* has also been associated with the canker diseases of tree species. However, the *Nectria* isolates in this study didn’t show their pathogenicity to the host plant. Therefore, the pathogenicity of *Nectria* had their specificity.

*Aspergillus* species could be sources of new medicines. For example, Kusari and Zhao had reported that an *Aspergillus* sp. was a source of anticancer medicines (Kusari et al. 2009; Zhao et al. 2009). Therefore, further research on the *Aspergillus* sp. isolated in this study may be interesting.

*Penicillium* is the source of penicillin, and recent results showed that endophytic *Penicillium* sp. had the capacity to secrete anti-tumor substances (Aly et al. 2010) or hypocrellin (Meng et al. 2011). We propose that the endophytic fungi isolated in this study from a medical plant are potential sources of medicines.

The growth-promotion factors and metabolites produced by endophytic fungi have been widely applied in medicine and agriculture. The most famous substance is taxol, a mitotic inhibitor used in cancer chemotherapy, which was originally produced by the yew tree and can be produced by endophytic fungi of yew trees (Rivera-Orduña et al. 2011). Similarly, a filtered liquid culture of endophytic fungi was analyzed to identify endophytes that could produce triterpenoid saponins. Overall, 19 of the isolated fungi showed a color reaction, which indicated that they could produce triterpenoid saponins.

### Table 2 Analysis of *Panax ginseng* triterpenoid saponins in typical isolates

| Isolate ID | Mean±Stdev (mg/ml) | Significance (P<0.05) | Closest species (Accession No.) |
|------------|--------------------|-----------------------|---------------------------------|
| Pg27       | 0.181±0.006        | a                     | *Fusarium subglutinans* (HQ876767) |
| Pg30       | 0.144±0.002        | b                     | *Aspergillus sydowii* (GU004536) |
| Pg42-1     | 0.144±0.009        | b                     | *Verticillium sp.* (HM057107)   |
| Pg33-2     | 0.136±0.004        | c                     | *Penicillium guttulatum* (HQ646592) |
| Pg34       | 0.133±0.002        | c                     | *Fusarium oxysporum* (FJ614650) |
| Pg41-2     | 0.130±0.002        | c                     | *Aspergillus fumigatus* (JQ268555) |
| Pg42-2     | 0.115±0.003        | d                     | *Nectria haematococca* (HM042416) |
| Pg50-1     | 0.109±0.004        | d                     | *Penicillium simplicissimum* (HM469430) |
| Pg61       | 0.079±0.004        | e                     | *Uncultured Ascomycota* (HQ33122) |
| Pg32       | 0.072±0.003        | f                     | *Penicillium guttulatum* (HQ646592) |
| Pg10       | 0.071±0.005        | f                     | *Uncultured Ascomycota* (HQ33122) |
| Pg41       | 0.063±0.003        | g                     | *Nectria haematococca* (HM042416) |
| Pg40       | 0.059±0.002        | gh                    | *Cladosporium cladosporioides* (JN651416) |
| Pg45       | 0.050±0.003        | hi                    | *Uncultured soil fungus* (EU691410) |
| Pg44       | 0.052±0.002        | ij                    | *Plectosphaerella cucumerina* (JF780520) |
| Pg36       | 0.048±0.003        | jk                    | *Fungal sp.* (GU552494)         |
| Pg12-1     | 0.042±0.003        | k                     | *Fusarium sp.* (AB294826)       |
| Pg31       | 0.035±0.003        | l                     | *Verticillium sp.* (AY312607)   |
| Pg14       | 0.023±0.002        | m                     | *Fusarium sp.* (AB294824)       |

### Table 3 Antimicrobial activity of representative endophytic fungi strains

| Test strains | Representative strains |
|--------------|------------------------|
|              | Pg27       Pg30       Pg42-1 |
| *Staphylococcus aureus* ACCC10499 | -           ++          -         |
| *Bacillus subtilis* ACCC10243    | -           -           -         |
| *Klebsiella pneumoniae* ACCC10498 | -           -           +++       |
| *Pseudomonas aeruginosa* ACCC10500 | -           -           -         |
| *Phytophthora cactorum* ACCC36421 | -           -           -         |
| *Rhizoctonia solani* ACCC36233    | ++          ++          -         |
| *Aspergillus niger* ACCC30005     | -           -           -         |
| *Fusarium sporotrichioides*       | -           -           -         |

Culture filtrate (0.2 ml) was added in each well (6 mm); (−) no inhibiton, (+) inhibition zone, +++ width of growth inhibition zone > 10 mm, ++ 5–10 mm, + 1-5 mm;
Figure 2 HPLC spectrums of culture filtrates from the representative strains. 1, Rg1; 2, Re; 3, Rf; 4, Rb1; 5, Rc; 6, Rb2; 7, Rb3; 8, Rd.
Among them, Pg27 (Fusarium sp.), Pg30 (Aspergillus sp.) and Pg42-1 (Verticillium sp.) exhibited higher concentrations of total saponins. These three isolates could be good candidates for further studies on their capacity to produce possible medical substances. *Fusarium* spp. were the main endophytes isolated from winter wheat (Sieber et al. 1988) and soybean (Pimentel et al. 2006). Many studies have shown that *Fusarium* spp. isolated from banana and tomato have the capacity to inhibit nematodes (Vu et al. 2004; Pocasangre et al. 1999; Hallmann and Sikora 1996). Phongpaichit et al. studied the antimicrobial activity of the endophytic fungi isolated from *Garcinia* species, with the results showing that the antimicrobial activities from different *Garcinia* species were different (Phongpaichit et al. 2006).

In the present study, several *Fusarium* spp. were isolated from the PG roots, and some could produce bioactive saponins. The concentrations of saponins from the different isolates were significantly different (*P* < 0.05), suggesting that their capacities to produce saponins were different. Further characterization of the bioactive compounds produced by fungi with high saponin-producing capacities could provide the possibility to obtain medicinal substances. HPLC results indicated that these three strains, as their host plants, had the capacity to produce some ginsenosides of *Panax ginseng*. Further research to improve their capacity of producing some ginsenosides is necessary. This work is under way.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
HW and HY performed most of the experimental work, took part in the evaluation of the results and wrote the manuscript. All authors participated in the design of the study, data collection and took part in the evaluation of the results. All authors read and approved the final manuscript.

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**References**
Aly AH, Debbab A, Kjer J, Proksch P (2010) Fungal endophytes from higher plants: a prolific source of phytochemicals and other bioactive natural products. Fungal Divers 41(1):1–16
Bayman P, Lebron LL, Tremblay RL (1997) Variation in endophytic fungi from roots and leaves of Lepontium (Orchidaceae). New Phytol 135(1):143–149. doi:10.1046/j.1469-8137.1997.00618.x
Danti R, Sieber TN, Sangwine G (2002) Endophytic mycobiota in bark of European beech (*Fagus sylvatica*) in the Apennines. Mycosc Res 106(11):1343–1348
De Siqueira WM, Conti R, De Araújo JM, Souza-Motta CM (2011) Endophytic fungi from the medicinal plant *Lippia sidoides* Cham. and their antimicrobial activity. Symbiosis 53(2):89–95
Dorworth CE, Macey DE, Sieber TN, Woods TAD (1996) Biological control of red alder (*Alnus rubra*) with indigenous pathogenic Ascomycotina. Can J Plant Pathol 18:315–324
Doty SL (2011) Growth-Promoting Endophytic Fungi of Forest Trees. Forestry Sci 80:151–156. doi:10.1007/978-94-007-1599-8_9
Guimarães DO, Borges WS, Kawano CY, Ribeiro PH, Goldman GH, Nomizo A, Thiemann OH, Oliiva G, Lopes NP, Pupo MT (2008) Biological activities from extracts of endophytic fungi isolated from *Viguiera stenostachya* and *Tithonia diversifolia*. FEBS J 521(1):134–144
Hallmann J, Sikora RA (1996) Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-borne pathogenic fungi. Eur J Plant Pathol 102(2):155–162
Hormazabal E, Piontelli E (2009) Endophytic fungi from chilean native gymnosperms: antimicrobial activity against human and phytopathogenic fungi. World J Microbiol Biotechnol 25:813–819
Kumaras RS, Kim HJ, Hur BK (2010) Tacol promoting fungal endophyte. *Pestalotiopsis* species isolated from *Taxis cupressoides*. J Biosci Bioeng 110(5):541–546
Kusari S, Lamshift M, Speltiee M (2009) Aspergillus fumigatus Fresenius, an endophytic fungus from Junipers communis L, Horstmann as a novel source of the antiprodrug deoxyphopholothion. J Appl Microbiol 107(3):1019–1030
Li H, Shen M, Zhou Z, Li T, Wei Y, Lin L (2012) Diversity and cold adaptation of endophytic fungi from five dominant plant species collected from the Baima Snow Mountain, Southwest China. Fungal Divers 54(1):79–86. doi:10.1007/s13225-012-0153-1
Lin X, Huang YJ, Su WJ, Qian XM, Shen YM (2010) Endophytes from the pharmaceutical plant, *Annona squamosa* isolation, bioactivity, identification and diversity of its polyketide synthase gene. Fungal Divers 41(1):41–51
Lin X, Lu C, Huang YJ, Zheng ZS, Su WJ, Shen YM (2007) Endophytic fungi from a pharmaceutical plant, *Campotheca acuminata* isolation, identification and bioactivity. World J Microbiol Biotechnol 23(7):1037–1040
Liu HG, Li T, Zhao YL, Zhang J, Wang YZ (2011) Determination of some metabolites of *Cordyceps sobolifera*. Afr J Microbiol Res 5(30):1558–1522
Lupo S, Tiscornia S, Bertiucci L (2001) Endophytic fungi from flowers, capsules and seeds of *Eucalyptus globulus*. Rev Iberoam Microl 18:38–41
Meng L, Sun P, Tang H, Li L, Diaseger S, Schulte B, Koehn KH, Hussain H, Zhang WY, Yi Y (2011) Endophytic fungus *Penicillium chrysogenum*, a new source of hypocrellins. Biochem Sys Ecol 39:163–165
Owensby GW, Gwinn KD, Vega FE (2010) Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl 55:113–128. doi:10.1007/978-90-481-3966-8_9
Park SU, Lim H, Park K, Park Y, Baer H (2012) Fungal endophytes from three cultivars of *Panax ginseng* meyer cultivated in Korea. J Ginseng Res 36(1):107–113
Phongpaichit S, Rujndijamal N, Rukachaisirikul V, Sakarajun J (2006) Antimicrobial activity in cultures of endophytic fungi isolated from *Garcinia* species. FEMS Immunol Med Microbiol 48(3):367–372
Pimentel IC, Glienke-Blanco C, Gabardo J, Stuart RM, Azevedo JL (2006) Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merril) under different environmental conditions. Braz J Arch Biol Techn 49(5):705–711
Pocasangre L, Sikora RA, Vilich V (1999) Schuster R-P: Survey of banana endophytic fungi from central America and screening for biological control of *Rodopholus similis*. In: Blanke M, Pohlan J (eds) The 2nd ISHS conference on Fruit production in the Tropics and Subtropics. Bonn-Rottgen, Germany, pp. 283–289
Redecke D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80
Rivera-Orduña FN, Suarez-sanchez RA, Flores-Bustamante ZR, Gracida-Rodriguez MN, Flores-Cotera LB (2011) Diversity of endophytic fungi of *Acer truncatum* and their role in decomposition. Fungal Divers 47:65–74. doi:10.1007/s13225-012-0160-2
Schulza B, Boyle C (2005) The endophyte continuum. Mycol Res 109(6):661–664
Sieber T, Riesen K, Müller E, Fried PM (1988) Endophytic Fungi in Four Winter Wheat Cultivars (*Triticum aestivum* L). Differing in Resistance Against *Stagonospora nodorum* (Berk.) Cast. & Germ. = *Septoria nodorum* (Berk.) Berk. J Phytopathol 122(4):289–306
Sun X, Guo LD, Hyde KD (2011) Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. Fungal Divers 47(1):85–95. doi:10.1007/s13225-010-0086-5
Suto M, Takebayashi M, Saito K, Tanaka M, Yokota A, Tomita F (2002) Endophytes as producers of xylanase. J Biosci Bioeng 93(1):88–90
Tadych M, Bergen MS, Johnson-Cicalese J, Polashock JJ, Vorsa N, White JF (2012) Endophytic and pathogenic fungi of developing cranberry ovaries from flower to mature fruit: diversity and succession. Fungal Divers 54(1):101–116. doi:10.1007/s13225-012-0162-2
Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10):2731–2739

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acid Res 25:4876–4882

Vu TT, Sikora RA, Hauschid R (2004) Effects of endophytic Fusarium oxysporum towards Radopholus similis activity in absence of banana. Commun Agric Appl Biol Sci 69(3):381–385

Xing X, Guo S, Fu J (2010) Biodiversity and distribution of endophytic fungi associated with Panax quinquefolium L. cultivated in a forest reserve. Symbiosis 51(2):161–166

Xu LL, Han T, Wu JZ, Zhang QY, Qin LP (2009) Comparative research of chemical constituents, antifungal and antitumor properties of the extracts of Panax ginseng and its endophytic fungus. Phytomedicine 16:609–616

Yang HY, Gao LJ, Wang XF, Wang WD, Cui ZJ (2007) Effects of cultivation conditions on the diversity of microorganisms involved in the conversion of rice straw to fodder. J Environ Sci 19(1):67–73

Yang RY, Feng PY, Li Q (2006) Research advances on the activity of endophyte pesticides. Agrochemicals 45(7):440–444

Zhao K, Ping W, Li Q, Hao S, Zhao D, Gao T, Zhou D (2009) Aspergillus niger var. taxi, a new species variant of taxol-producing fungus isolated from Taxus cuspidata in China. J Appl Microbiol 107(4):1202–1207

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