J Ginseng Res 43 (2019) 77–85

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research article

The effect of *Glomus intraradices* on the physiological properties of *Panax ginseng* and on rhizospheric microbial diversity

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ARTICLE INFO

Article history:
Received 11 February 2017
Received in Revised form
8 August 2017
Accepted 14 August 2017
Available online 19 August 2017

Keywords:
arbuscular mycorrhizal fungi
bacteria
fungi
Panax ginseng

ABSTRACT

Background: *Glomus intraradices* is a species of arbuscular mycorrhizal fungi that, as an obligate endomycorrhiza, can form mutually beneficial associations with plants. *Panax ginseng* is a popular traditional Chinese medicine; however, problems associated with ginseng planting, such as pesticide residues, reduce the ginseng quality.

Methods: In this experiment, we studied the effect of inoculating *G. intraradices* on several physiological properties and microbial communities of ginseng. UV-Visible Spectrum method was used to detect physical properties. Denaturing gradient gel electrophoresis method was used to analyze microbial communities.

Results: The results indicated that inoculation with *G. intraradices* can improve the colonization rate of lateral ginseng roots, increase the levels of monomeric and total ginsenosides, and improve root activity as well as polyphenol oxidase and catalase activities. We also studied the bacterial and fungal communities in ginseng rhizospheric soil. In our study, *G. intraradices* inoculation improved the abundance and Shannon diversity of bacteria, whereas fungi showed a reciprocal effect. Furthermore, we found that *G. intraradices* inoculation might increase some beneficial bacterial species and decreased pathogenic fungi in rhizospheric soil of ginseng.

Conclusion: Our results showed that *G. intraradices* can benefit ginseng planting which may have some instructive and practical significance for planting ginseng in farmland.

1. Introduction

Panax ginseng Meyer is a popular traditional Chinese medicine [1]. Many studies using animal models have shown that ginseng plays an important role in enhancing the immune response and inhibiting bacterial quorum sensing [2]. With the improvement of people's living standards, the need for *P. ginseng* is increasing in recent years. To meet this demand, the scale of ginseng planting has rapidly increased in recent years. However, the limited availability of planting soil and constant farming obstacles have restricted ginseng planting [3]. Furthermore, the utilization of fungicides and chemical fertilizers has decreased the quality of soil and ginseng, which could seriously affect human health.

Mycorrhiza are symbiotic associations between mycorrhizal fungi and a host plant [4]. It is one of the most common symbiosis on earth, with 80% of plants having mycorrhiza. In addition, the mycorrhizal hyphae present in soil can account for a quarter of the total biomass in soil [5]. Arbuscular mycorrhizal fungi (AMF) are a group of endomycorrhiza that perform an important ecosystem function by improving the ability of plants to capture nutrients from soil. Research has revealed that plants associated with AMF grow faster than the non-AMF-associated plants [6]. According to

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http://dx.doi.org/10.1016/j.jgr.2017.08.005
p1226-8453 e2093-4947/$ – see front matter

reports of AMF application to host plants, AMF are able to improve the nutrient absorption of the host plants by generating long hyphae in the soil and by providing increased resistance to environmental stresses [7,8]. One essential function of the AMF symbiosis with the host plants is the bidirectional exchange of nutrients. AMF enhance plant nutrient acquisition by exchanging phosphorus (P) captured from soil for carbon (C) in the form of photosynthate [9,10]. Thus, the application of AMF to medicinal plants is of some interest to researchers.

The microbial community is an important component of soil. Some research has shown that AMF also affect the community and activity of soil microbiome. For instance, Nuccio et al. [11] found that the phylum of *Firmicutes* was correlated with the AMF positively. The relative abundance of *Firmicutes* with AMF inoculation was increased significantly in comparison with that without AMF inoculation. In contrast, it indicated a negative correlation between AMF and the phylum of *Actinobacteria* or *Comamonadaceae*, and these two phyla had a lower abundance with AMF inoculation [11]. Xavier and Germida [12] found that *Bacillus* sp. can be isolated from AMF spores and have the ability to promote the germination and colonization of AMF spores. Roots colonized by AMF can be afforded protection from invasion by fungal pathogens [13]. However, little research has been done regarding the application of AMF to ginseng despite it being well-known symbiont of this plant.

Previously, some studies have been conducted regarding the effects of inoculating AMF on ginseng planting. Fournier et al. [14] demonstrated that *G. intraradices* inoculation could promote the growth and ginsenoside of *Panax quinquefolius*. Thomas [15] showed that inoculating *G. intraradices*, *Glomus desserticcola*, and *Glomus mosseae* could improve the growth of *Panax quinquefolius*. Cho et al. [16] studied the effect of AMF inoculation on ginseng seedlings and found that it improved nutrient absorption and root weights. However, there have been no studies demonstrating the effect of AMF inoculation on stress-related physiological properties and corresponding ginsenoside content of *P. ginseng* as well as the change of rhizospheric microbial communities and diversities.

G. intraradices is one of the most common species of AMF identified in the natural ecosystems, and it harbors in most of the plants as a symbiotic fungus [4]. *G. intraradices* can dominate arbuscular mycorrhizal communities in heavily textured agricultural soil [17]. Accordingly, here we used *P.* ginseng and *G. intraradices* to construct the AM symbiosis in order to determine the effect of AMF on ginseng growth. We hypothesized that AMF can (1) promote some stress-related physiological indexes and ginsenoside content and (2) improve the rhizospheric microbial community of *P. ginseng*.

2. Materials and methods

2.1. Plant and soil materials

Ginseng seedlings (2 yrs old and of same size) were selected in the autumn at the herbal garden experimental station of the Northeast Institute of Geography and Agroecology in Jilin Province, China (125°23′44″E, 43°59′58″N). The seedlings were cleaned and sterilized with sodium hypochlorite for 10 min, rinsed with tap water, and transplanted to a new seedling bed. Six rows of ginseng were planted in a small bed, each having five plants, and the rows were 20 cm apart from each other. The bed soil was sandy loam with the following properties: a pH of approximately 6.7, 80.9 mg/kg of available P, 110 mg/kg of available N, 41.4 mg/kg of available K, and 1.61 g/kg of organic matter. AMF species of the original soil taken from 2-yr-old ginseng were analyzed by high-throughput sequencing. There are seven species of AMF, namely *Corymbiglomus* sp., *Claroideoglomus* sp., *Funneliformis* sp., *Glomus* sp., *Rhizophagus* sp., *Septoglomus* sp., and one unclassified species in the original soils. However, the abundance of the total AMF was only 0.2% of the total fungi (Fig. S1). The Illumina sequencing data in the present study has been deposited into NCBI SRA database with the accession number as SRP114909.

2.2. Spores inoculated on ginseng root

The *G. intraradices* spores were obtained from soils of *Medicago sativa* cultivated in pots that had been inoculated with *G. intraradices*. Spores were collected from the *Medicago sativa* mycorrhizosphere using the wet-sieving method by placing the mycorrhizosphere in a 45-μm mesh sieve and rinsing them vigorously with cold tap water. The spores remained on the sieve with larger soil particles, which were then isolated by placing the sample in 50% glycerol and centrifuging at 5000 × g for 5 min. The cleaned spore preparation was examined microscopically (Fig. S2), and subsequently 30 spores were inoculated into 1 g of sterilized roseite. Each plant was treated with 2 g of inoculated roseite, and the control group was treated with 2 g of sterilized roseite. Each group had a bed of three rows with five ginseng plants in each row as described above. The plants were grown in the seedling bed for 2 yrs and harvested in September.

2.3. Colonization rates and total glomalin-related soil protein content

After harvesting, the lateral ginseng roots were cut and used to test the colonization rates by the trypan blue method [18]. The 1-cm lateral root sections were fixed with Carnoy's fluid for 20 min, rinsed thoroughly with water, and then stained in 10% potassium hydroxide (KOH; Xilong, Guangdong, China) in a 90°C water bath for 1 h. The KOH solution was discarded, and the roots were rinsed thoroughly with water. After being dipped in alkaline H_2O_2 (Xilong, Guangdong, China) for 20 min, the roots were dipped in 5% lactic acid (Guangfu Fine Chemical Research Institute, Tianjin, China) for 3–4 min. The roots were then dipped into trypan blue dye liquid (Dingguo, Beijing, China) in a 90°C water bath for 30 min. The roots were then rinsed with a lactic acid-glycerinum solution to clear the dye and were observed under a microscope to determine the colonization rate.

Rhizospheric soil was collected from ginseng lateral roots, and total glomalin-related soil protein (GRSP) content was detected by the sodium citrate extraction method. In detail, 1 g samples of dry-sieved 2 mm soil were extracted with 2 mL 50mmol/L citrate (Beijing Chemical Works, Beijing, China), pH 8.0 at 121°C for 90 min. The supernatant was obtained by centrifugation at 10,000 *g* for 5 min and transferred to a 50-mL triangular flask. GRSP was repeatedly extracted from the residue with the same extraction condition for several times until no red-brown color was shown in the supernatant. Next, the mixed supernatant was centrifuged to remove the soil particles (10,000 *g* for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin (Jinsul Bio-Technology, Shanghai, China) as the standard.

2.4. Detection of root activities, weight increment of root, catalase and polyphenol oxidase activities

Root activity was determined by the triphenyl tetrazolium chloride method as described by Zhang et al. [19]. Weight increment of fresh ginseng root was calculated by the values that harvested root weight minus original root weight. The values of root weight were the average values of the three replicates with five ginseng roots for each replicate in inoculated (+AMF) and non-inoculated (−AMF) groups as described in Section 2.2. Catalase

activity was determined by the potassium permanganate titration method, as described by Cohen et al. [20]. Polyphenol oxidase activity was determined by the 2, 3-dihydroxy-L-phenylalanine method, as described by Holzapfel et al. [21].

2.5. Detection of ginsenoside monomers and total ginsenoside

The main ginseng roots were carefully washed with tap water and dried completely in an oven at 60°C. The roots were then ground into a powder which was then passed through a 69-μm mesh sieve. A total of 0.50 g of the powder was then packed into filter paper, which was placed into a Soxhlet extractor with 100 mL of 100% HPLC-grade methanol (Yuwang Chemical Inc., Shandong, China) in the extraction chamber. The Soxhlet extractor ran in a 90°C water bath for 12 h. The extracting solution was then dried by rotary evaporators (RE-52A, Yarong, Shanghai, China) with a vacuum pump at 50°C. Next, the residue was redissolved by adding 8 mL 50% methanol (Yuwang Chemical Inc., Shandong, China) to a round-bottom flask and was brought to a final volume of 10 mL with 50% methanol in a volumetric flask.

The ginsenoside content was determined using a reversed-phase HPLC Spectra system 2489 (Waters, Milford, USA) with a spherical C_{18} reverse phase column (4.6 mm × 250 mm, 5 μm). The mobile phase solution was (A) acetonitrile (HPLC grade; Fisher Chemical, Pittsburgh, USA) and (B) water. The program used was as follows: 0–20 min, 22% acetonitrile; 20–25 min, 22–30% acetonitrile; 25–45 min, 30–46% acetonitrile; 45–55 min, 46–64% acetonitrile; 55–70 min, 64–66% acetonitrile; 70–85 min, 66–100% acetonitrile. The peaks were detected by UV spectrophotometry at 205 nm [22]. Ginsenoside standards included Rg1, Re, Rb1, Rc, Rb2, and Rd (National Institute for Food and Drug Control, Beijing, China). Qualitative identification of ginsenoside peaks was determined by cochromatography (equivalent retention time) with chemically pure standards, and quantitative analysis of ginsenoside was based on the area integration of the peak in samples compared with a standard curve [22]. Ginsenoside standards, Rg1, Re, Rb1, Rc, Rb2, and Rd were mixed together and made into concentrations of 0.05 μg/mL, 0.1 μg/mL, 0.5 μg/mL, 1.0 μg/mL, 2.0 μg/mL, 5.0 μg/mL for linear-regression analysis of each ginsenoside standards.

2.6. Analysis of bacterial and fungal diversities based on PCR-denaturing gradient gel electrophoresis

Soil DNA was extracted with the FastDNA™ SPIN Kit for soil as recommended by the manufacturer (MP Biomedicals, CA, USA, code No. 116560200). Samples were prepared for PCR-denaturing gradient gel electrophoresis (DGGE). The 16S bacterial rDNA V3 region was amplified using the primers GC-338F (CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG) and 518R (ATTACCGCGGCTGCTGG). PCR was performed in a LifePro PCR amplifier (TC-96/G/H/(b) A, BIOER, Hangzhou, China) using the following parameters: an initial denaturation for 4 min at 94°C, followed by 35 amplification cycles of denaturation (94°C for 50 s), annealing (54°C for 50 s), and extension (72°C for 50 s), with a final extension at 72°C for 10 min. The fungal 18S rDNA region was amplified using the primers GC-Fung (CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCATTCCCCGTTACCCGTTG) and NS1 (GTAGTCATATGGTTGTCTC) with the reaction parameters as follows: an initial denaturation for 4 min at 94°C; followed by 35 amplification cycles of denaturation (94°C for 50 s), annealing (52°C for 50 s), and extension (72°C for 55 s), with a final extension at 72°C for 10 min. The PCR products were checked by agarose gel electrophoresis (Invitrogen, California, USA).

The final PCR products were separated by DGGE using the Junyi mutation detection system (Junyi Laboratories, Beijing, China). The PCR products were applied to 8% (m/v) polyacrylamide gels in 1 × TAE buffer with a 40–60% gradient for bacteria and a 20–40% gradient for fungi. Electrophoresis was performed at 80 V for 12 h at a constant temperature of 60°C. DNA was stained with 5mmol/L ethidium bromide (Dingguo, Beijing, China) for 30 min and then visualized using an UV imager (GenoSens, Shanghai, China). The separated bands were visible under UV light. The presence of the same horizontal bands indicated the same bacterial species. Bands with different sizes from each group were selected for gel extraction. The DNA was purified using an AxyPrep DNA Gel Extraction Kit (Axygen, Suzhou, China). The isolated PCR products were then sent to Sangon Biotech (Sangon Biotech, Shanghai, China) for sequencing. The obtained sequences were blasted against the GenBank database and submitted to GenBank under the following accession numbers (KY568004–KY568010 for bacteria and KY605252–KY605253 for fungi). Using bacterial sequences with a high similarity, we constructed a phylogenetic evolutionary tree with the MEGA 5.1 software using the neighbor-joining method with 1000 bootstraps [23].

2.7. Statistical analysis

One-way ANOVA was used for analyzing the colonization rates and GRSP content with a Duncan test using SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA). The Shannon and abundance indexes of bacteria and fungi were calculated using the vegan package 2.4.1 in R software version 3.2.1 (https://www.r-project.org). The DGGE bands were analyzed with the Quantity One 4.5 software (Bio-Rad Laboratory, Hercules, CA, USA). A principal component analysis (PCA) was carried out using the PCA function within the FactoMineR package 1.36 in R software version 3.2.1 (https://www.r-project.org), and the graph was drawn by the ggplots package of R. The Bray–Curtis distances were determined by the distance function using the vegan package 2.4.1 in R software version 3.2.1 (https://www.r-project.org). To determine whether the diversity of the bacterial and fungal communities, as well as that of AMF, affected the composition of the original community, we determined Bray–Curtis distances using the Permutational multivariate analysis of variance test (PERMANOVA) using the adonis function in the vegan package in R software version 3.2.1 (https://www.r-project.org) [24].

3. Results

3.1. Colonization rates, microscopic structures of roots colonized with AMF, rhizospheric GRSP content, and representative phenotype of ginseng root analysis

To analyze whether inoculating AMF can significantly colonize ginseng roots, colonization rates for the lateral ginseng roots were tested by the trypan blue method as described above. The results showed that the colonization rate was 40.33% for the inoculated ginseng roots, which was significantly higher than non-inoculated ginseng roots (Fig. 1A). The microscopic structures of colonized lateral root in +AMF group had clear vesicle structure (Fig. 1B), and the non-colonized lateral root in −AMF group did not have the vesicle structure (Fig. 1C). In addition, colonized lateral root in the −AMF group had fewer vesicle structures (Fig. 1D) than that in +AMF ginseng root (Fig. 1B).

The GRSP content in the rhizospheric soil was tested and determined to be 23.42 mg/g in the +AMF group, which was significantly higher than that of the −AMF group, with the value of 4.87 mg/g (Fig. 2A). The representative phenotype of ginseng root could clearly show the longer and more lateral roots in +AMF group than in −AMF group (Fig. 2B).

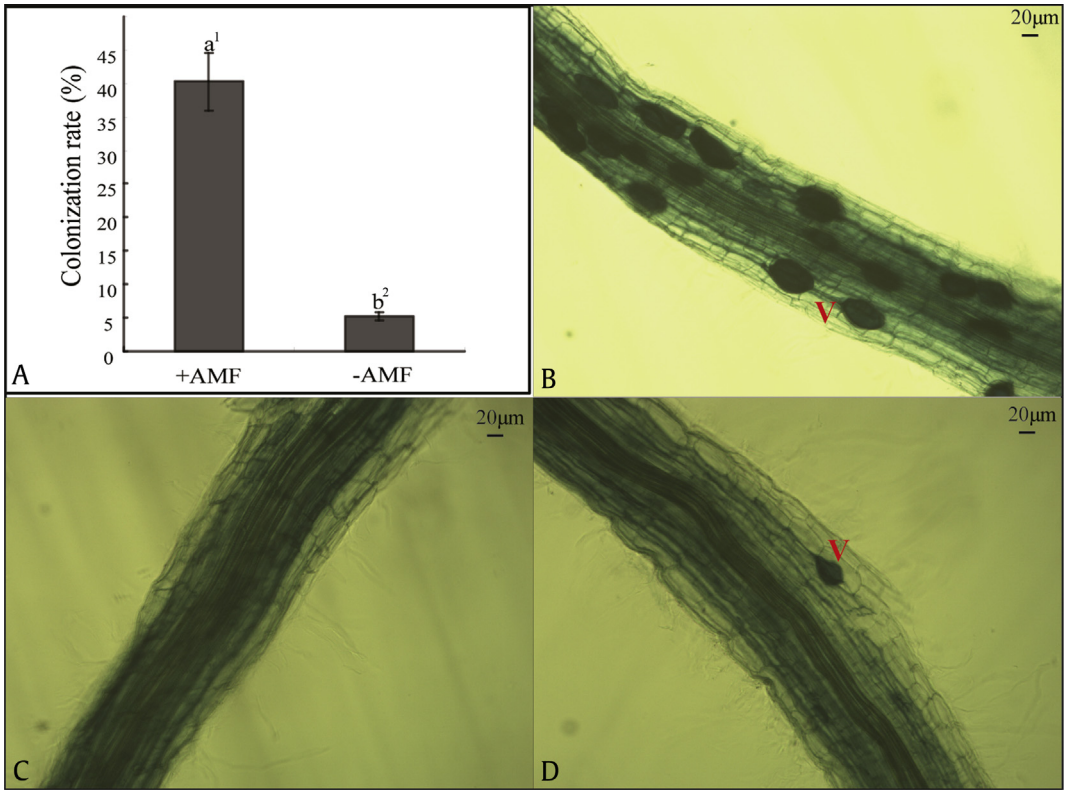

Fig. 1. The comparison of the (AMF) colonization rate and microscopic structures of roots colonized with AMF. (A) The comparison of the AMF colonization rate between inoculated (+AMF) and non-inoculated (−AMF) ginseng lateral roots. X-axis and Y-axis indicate group and colonization rate, respectively. Error bars indicate standard errors. The experiment was performed in triplicate (two technical repeats for each biological replicate). Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. (B) The microscopic vesicle structures in roots colonized with AMF were shown as +AMF group. (C) The microscopic structures of the roots without the AMF colonization were shown as −AMF group. (D) The roots with the AMF colonization were shown as −AMF group. [1)] Significantly different, $p < 0.05$. [2)] Significantly different, $p < 0.05$. AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance; V, vesicle structure.

3.2. Root activity, weight increment of roots, polyphenol oxidase and catalase activity tests of +AMF and −AMF groups

To analyze the effect of AMF on the growth and stress-resistance index of ginseng seedlings, root activity, weight increment of ginseng root, polyphenol oxidase and catalase activities of lateral roots were tested. The results showed that root activity was 0.072 mg/(g · h) in the +AMF group, significantly higher than that in −AMF group (Fig. 3A). As shown in Fig. 3B, inoculating AMF had improved the values of weight increment of ginseng root compared with non-inoculated AMF (with 6.59% of plant growth rate). However, the result was not significant by one-way ANOVA

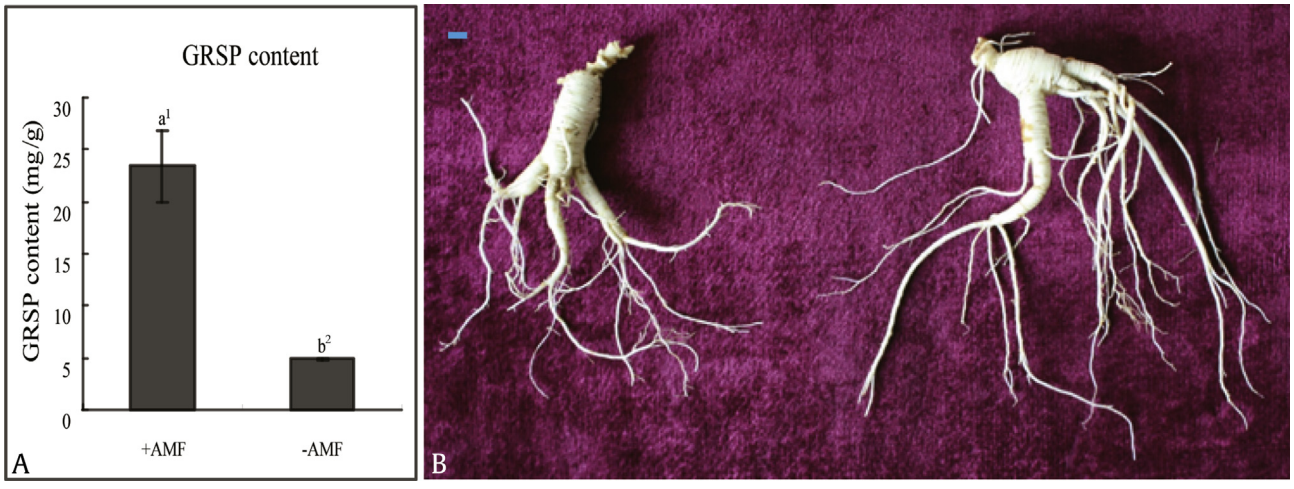

Fig. 2. GRSP contents and phenotype of ginseng roots in inoculated (+AMF) and non-inoculated (−AMF) groups. (A) GRSP contents of ginseng rhizospheric soil in +AMF and −AMF groups were measured by sodium citrate extraction method. The experiment was performed in triplicate (two technical repeats for each biological replicate). X-axis, Y-axis indicate group and GRSP contents, respectively. (B) The phenotype of ginseng root in +AMF and −AMF groups. Error bars indicate standard errors. The experiment was performed in triplicate. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. Blue line was scale bar with 0.5 cm. [1)] Significantly different, $p < 0.05$. [2)] Significantly different, $p < 0.05$. AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance; GRSP, glomalin-related soil protein.

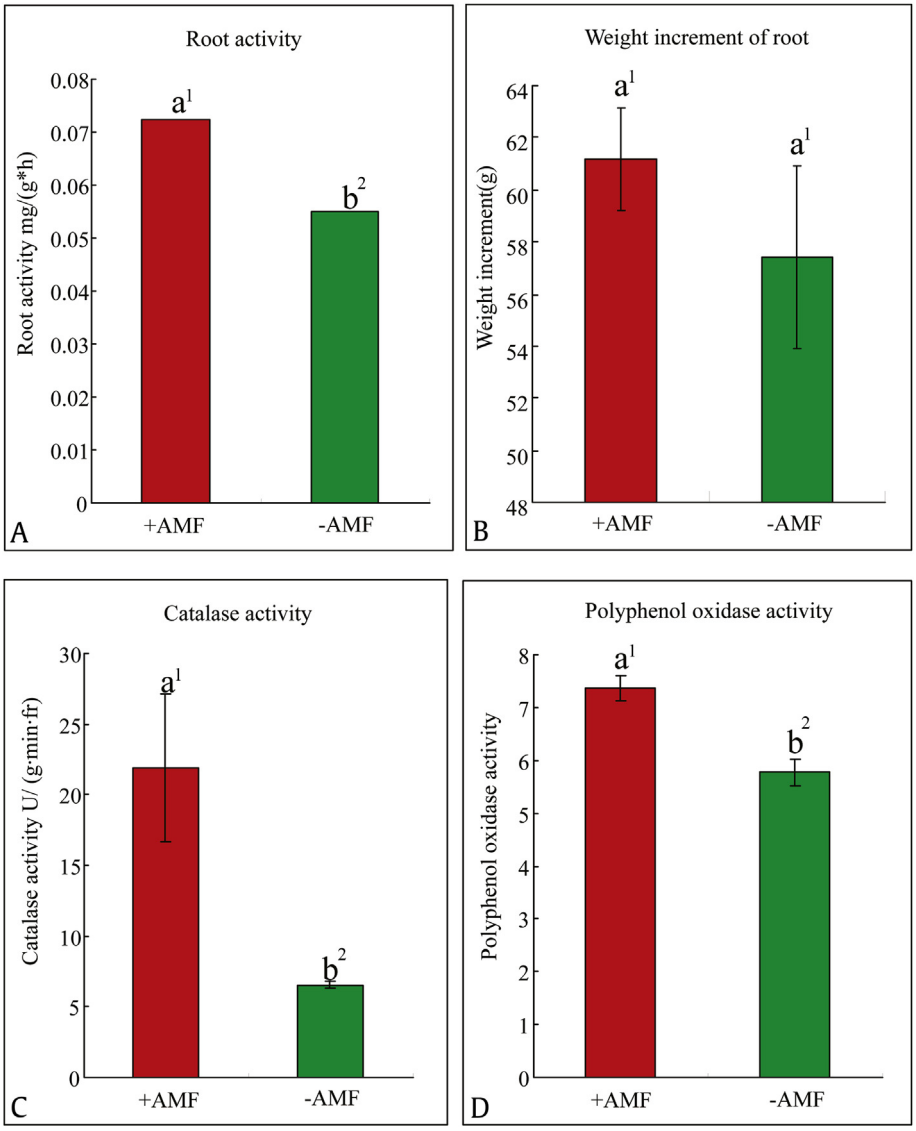

Fig. 3. Root activity, weight increment of roots, polyphenol oxidase and catalase activity tests. (A) Root activity was determined by the triphenyl tetrazolium chloride method in inoculated (+AMF) and non-inoculated (−AMF) groups. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. The experiment was performed in triplicate (two technical repeats for each biological replicate). X-axis and Y-axis indicate group and root activity, respectively. (B) Weight increment of root was calculated as the values that harvested weight minus original weight. The values of root weight were the average values of the three replicates with five ginseng roots for each replicate in +AMF and −AMF groups. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. The experiment was performed in triplicate. X-axis and Y-axis meant group and weight increment of root, respectively. (C) Catalase activity was determined by the potassium permanganate titration method. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. The experiment was performed in triplicate (two technical repeats for each biological replicate). The experiment was performed in triplicate. X-axis and Y-axis indicate group and catalase activity, respectively. (D) Polyphenol oxidase activity was determined by the 2, 3-dihydroxy-L-phenylalanine method. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. The experiment was performed in triplicate (two technical repeats for each biological replicate). X-axis and Y-axis indicate group and polyphenol oxidase activity, respectively. [1)] Significantly different, $p < 0.05$. [2)] Significantly different, $p < 0.05$. AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance.

($p > 0.05$). As for polyphenol oxidase and catalase activity, the catalase activity was 21.93 U/ (g·min·fr) in the +AMF group, much higher than that in the −AMF group (Fig. 3C); and the polyphenol oxidase activity was 7.37 in the +AMF group, significantly higher than that in the −AMF group (Fig. 3D).

3.3. *Ginsenoside monomers and total ginsenoside analysis of +AMF and −AMF groups by HPLC method*

The presence of ginsenoside monomers Re, Rg1, Rb1, Rb2, Rc, and Rd was tested using an HPLC method. The HPLC curve of 2 μg/mL standard ginsenoside monomers is shown in Fig. S3. The peak shape of Re, Rg1, Rb1, Rb2, Rc, and Rd was sharp and could be identified clearly. The sequence of the occurred peaks for ginsenoside monomers was Rg1, Re, Rb1, Rc, Rb2, and Rd with each retention time at 15.41 min, 16.34 min, 33.82 min, 34.73 min, 35.71 min, and 37.85 min, respectively (Fig. S3). After calculating by linear regression from the peak area of each ginsenoside monomer, the content of ginsenoside monomer in samples was obtained as shown in Table 1. As shown in Table 1, the levels of all of the ginsenoside monomers in the +AMF group were significantly higher than those of the −AMF group. Specifically, the contents of

Table 1
Ginsenoside content of ginseng root in inoculated (+AMF) and non-inoculated (−AMF) groups

Ginsenoside content	Re	Rg1	Rb1	Rb2	Rc	Rd	TR
−AMF	2.12b	3.02b	7.08b	1.63b	8.31b	1.14b	23.31b
+AMF	3.21a	4.24a	9.61a	2.23a	10.14a	1.68a	31.11a

Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$
AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance; TR, total ginsenoside

ginsenoside monomers Re, Rg1, Rb1, Rb2, Rc, and Rd were 3.21 mg/g, 4.24 mg/g, 9.61 mg/g, 2.23 mg/g, 10.14 mg/g, and 1.68 mg/g, respectively, in the +AMF group, which had improved by 51.26%, 40.14%, 35.73%, 36.81%, 22.02%, and 47.36%, respectively, compared with those in the −AMF group. Total ginsenoside is reported as the sum of the six ginsenoside monomers. The content of total ginsenoside in the +AMF group was 31.10 mg/g, which had improved by 33.48% over the −AMF group, with the value of 23.31mg/g (Table 1).

3.4. Changes of bacterial and fungal communities and diversities in rhizospheric soil of ginseng root by inoculating AMF

Based on the PCR-DGGE analysis (Figs. 4A and 5A), we tested the bacterial and fungal communities and diversities. Using PCA and PERMANOVA tests, we observed that the replicates clustered together and that the bacterial and fungal communities were significantly different between +AMF and −AMF groups (Fig. S4), indicating that the repeatability of the results was good. As for bacterial PCR-DGGE analysis, there were total 23 bands in all the samples (Fig. 4A). There were more bands in +AMF group. Some bands were specific to the +AMF or −AMF group. Two bands only occurred in the −AMF group, and five bands only occurred in the +AMF group. Other 16 bands were shared in +AMF and −AMF groups. The sequences of the specific bands were blasted against the GenBank database, and the sequences with the highest homology in GenBank were selected to construct a phylogenetic tree (Fig. 6A). The results showed that the two bands specific to the −AMF group are homologous to *Pseudomonas aeruginosa* and an uncultured *Erwinia* sp. The five bands specific to the +AMF group are homologous to an uncultured *Bacillus* sp., uncultured *Flavobacterium* sp., uncultured *Rhizobium* sp., and uncultured bacterial species of unknown genera. As shown in Fig. 4B, the Shannon and abundance indexes of the +AMF group were 18.66 and 2.89, respectively, which were significantly higher than those of the −AMF group, which were 11.00 and 2.35 respectively.

The fungal community and diversity were also analyzed by the PCR-DGGE method (Fig. 5A). There were more bands in the −AMF group. In total, there were eight bands in all the samples, with six bands shared in +AMF and −AMF groups and two bands specific to the −AMF group (Fig. 5A). The sequences of the specific bands were also blasted against the GenBank database, and the sequences with the highest homology in GenBank were selected to construct a phylogenetic tree (Fig. 6B). From phylogenetic tree analyses, we found that the two bands are highly homologous to *Fusarium* sp. and *Alternaria* sp (Fig. 6B). As shown in Fig. 5B, the Shannon and abundance indexes of the +AMF group were 5.33 and 1.47, respectively, significantly lower than those of the −AMF group, which were 8 and 1.94.

4. Discussion

Ginseng is one of the many species of mycorrhizal plants [25]. There have been numerous studies regarding AMF communities on ginseng roots. Many kinds of AMF spores in field soil can colonize ginseng roots. In this study, we observed vesicle structures in some lateral roots of the non-inoculated plant group, demonstrating that ginseng is a mycorrhizal plant that can be colonized by AMF in natural soils. The results agree with those of Sohn et al.'s [26] study

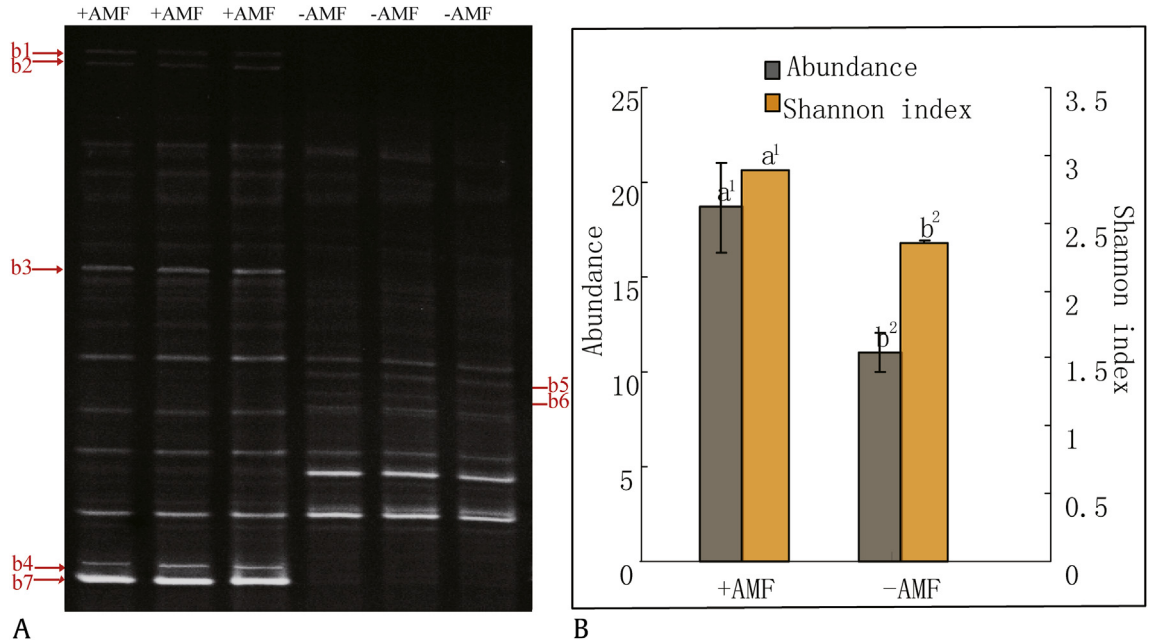

Fig. 4. Bacterial communities and diversities based on DGGE profiles in rhizospheric soil of ginseng roots in inoculated (+AMF) and non-inoculated (−AMF) groups. (A) Bacterial DGGE profiles of ginseng rhizospheric soil in +AMF and −AMF groups. (B) Bacterial abundance and Shannon diversity indexes of ginseng rhizospheric soil in +AMF and −AMF groups. Error bars indicate standard errors. The experiment was performed in triplicate. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. X-axis and Y-axis indicate group and bacterial abundance and Shannon diversity indexes, respectively. [1)] Significantly different, $p < 0.05$. [2)] Significantly different, $p < 0.05$. AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance. DGGE, denaturing gradient gel electrophoresis.

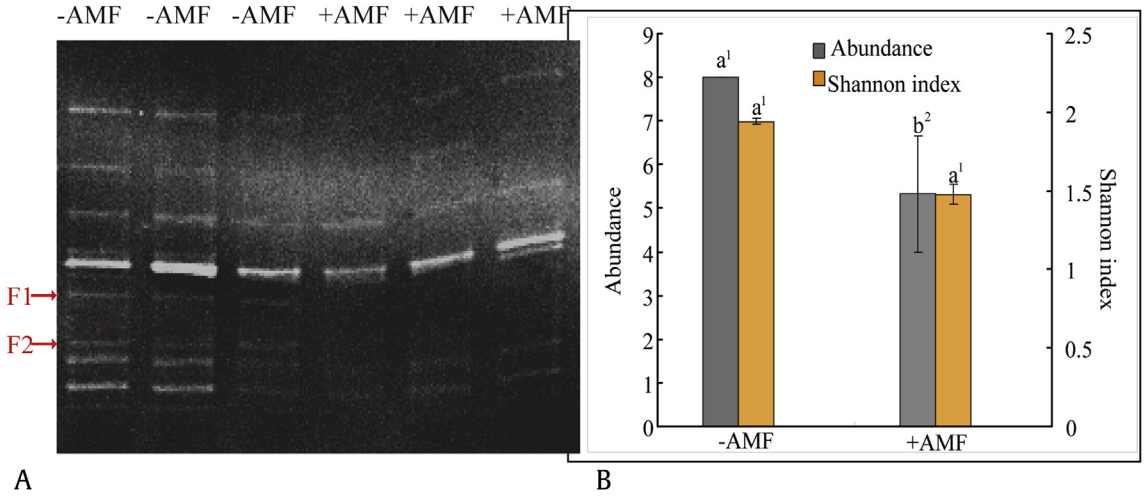

Fig. 5. Fungal communities and diversities based on DGGE profiles in rhizospheric soil of ginseng roots in inoculated (+AMF) and non-inoculated (−AMF) groups. (A) Fungal DGGE profiles of ginseng rhizospheric soil in +AMF and −AMF groups. (B) Fungal abundance and Shannon diversity indexes of ginseng rhizospheric soil in +AMF and −AMF groups. The experiment was performed in triplicate. Error bars indicate standard errors. Results were analyzed with one-way ANOVA and Duncan's multiple range tests. Values with different superscript numbers are significantly different, $p < 0.05$. X-axis and Y-axis indicate group and fungal abundance and Shannon diversity indexes, respectively. [1)] Significantly different, $p < 0.05$. [2)] Significantly different, $p < 0.05$. AMF, arbuscular mycorrhizal fungi; ANOVA, analysis of variance. DGGE, denaturing gradient gel electrophoresis.

on the distribution of AMF in ginseng cultivated fields in Jeonnam Province. However, the colonization rate increased significantly when we added *G. intraradices* spores to the soil. Our results demonstrated that addition of AMF spores can promote the colonization rate and shape the symbiosis with ginseng roots, which may benefit ginseng plants.

AMF can promote nutrient absorption of plants, such as P, K and N, and improve physiological indexes of plants [27]. The colonization of AMF can also increase the ability of plants to resist environmental stresses [28,29]. Thus, the application of AMF to soils is a possible solution for some difficult-to-cultivate plants. The typical environmental conditions, such as light, soil, and water, in which ginseng is planted are very harsh [30]. In this study, we found that inoculated AMF could improve root activity as well as polyphenol oxidase and catalase activities. Root activity is an important index for plants and represents metabolism in roots. Ghazanfar et al. [31] also showed that AMF can significantly enhance the root activity level of tomato plant under moderate NaCl stress. Polyphenol oxidase and catalase activities are indicative of the ability of a plant to resist adverse environmental stresses. Minton et al. [32] showed that AMF can significantly improve plant defenses by improving polyphenol oxidase activity. Thus, our results showed that AMF can likely improve the metabolic ability of ginseng roots and improve resistance to environmental stresses. However, we did not get the significant weight increment of root as described by Fournier et al. [14] and Cho et al. [16] which may be caused by different soil conditions compared with their studies.

Ginsenoside, an active component in ginseng, can improve people's health and exert a potent anticancer activity [33]. The ginsenoside monomers Re, Rg1, Rb1, Rb2, Rc, and Rd are the most common components in ginsenoside, and their sum represents total ginsenoside. Total ginsenoside is an indicator of ginseng quality. In this research, inoculation of ginseng with AMF improved the content of all ginsenoside monomers and thus the total ginsenoside content. An interesting phenomenon was observed in that inoculated AMF may improve nutrient absorption, which may affirm the importance of AMF in improving ginseng's quality. Silva et al. [34] found that AMF can increase the oleoresin production of micropropagated *Zingiber officinale* and consequently increase the aggregate of ginger rhizome production. This indicated that AMF can improve the medicinal component of medicinal plants.

Rhizobacteria and rhizospheric fungi play important roles in material and energy exchange between soil and plant. However, some bacteria and fungi are pathogens and cause many diseases to plants. In the process of ginseng planting, there are many diseases, such as rust rot disease and rot disease, caused by fungal pathogens such as *Alternaria panax*, *Fusarium oxysporum*, *Fusarium solani*, *Phoma herbarum*, and *Mycocentrospora acerina* [35]. However, there are few bacteria known to cause disease in ginseng. In addition, many bacteria such as *Bacillus* sp. and *Pseudomonas* sp. can promote ginseng growth by inhibiting pathogens and dissolving phosphate and potassium [36]. There is evidence that some bacteria can be isolated from AMF spores, which may affect the germination and hypha elongation [12]. Mechri et al. [37] reported that inoculated *G. intraradices* can significantly change microbial communities in the rhizosphere of olive trees (*Olea europaea* L.). In this study, we found that soil inoculated with AMF can increase the bacterial abundance and bacterial Shannon diversity and decrease fungal abundance and fungal Shannon diversity.

We also found that inoculated AMF improved the presence of *Bacillus* sp., *Flavobacterium* sp., and *Rhizobium* sp. *Bacillus* sp. are well known for promoting plant growth having an anti-pathogen effect in soil [38]. In addition, *Rhizobium* sp. are well-known nitrogen-fixing bacteria for their special cell structure [39]. In contrast, the non-inoculated group had two specific species, *Pseudomonas aeruginosa* and *Erwinia* sp. *Pseudomonas aeruginosa* is an opportunistic pathogen that may cause root rot to ginseng [40]. *Erwinia* sp. are a common disease-causing group that cause bacterial blight in plants and soft rot disease in ginseng [41]. Our results suggest that inoculation with AMF can inhibit the emergence of bacterial pathogens.

Sayeed and Zakia [42] reported that inoculation with *G. intraradices*, *Rhizobium* sp., and *Pseudomonas straita* could significantly promote plant growth and reduce the invasion of pathogens. In this study, only two specific species, *Fusarium* sp. and *Alternaria* sp., were observed in the non-inoculated group. *Fusarium* sp. and *Alternaria* sp. are common species in ginseng root rot disease [43,44]. Thus, the results of this study are in agreement

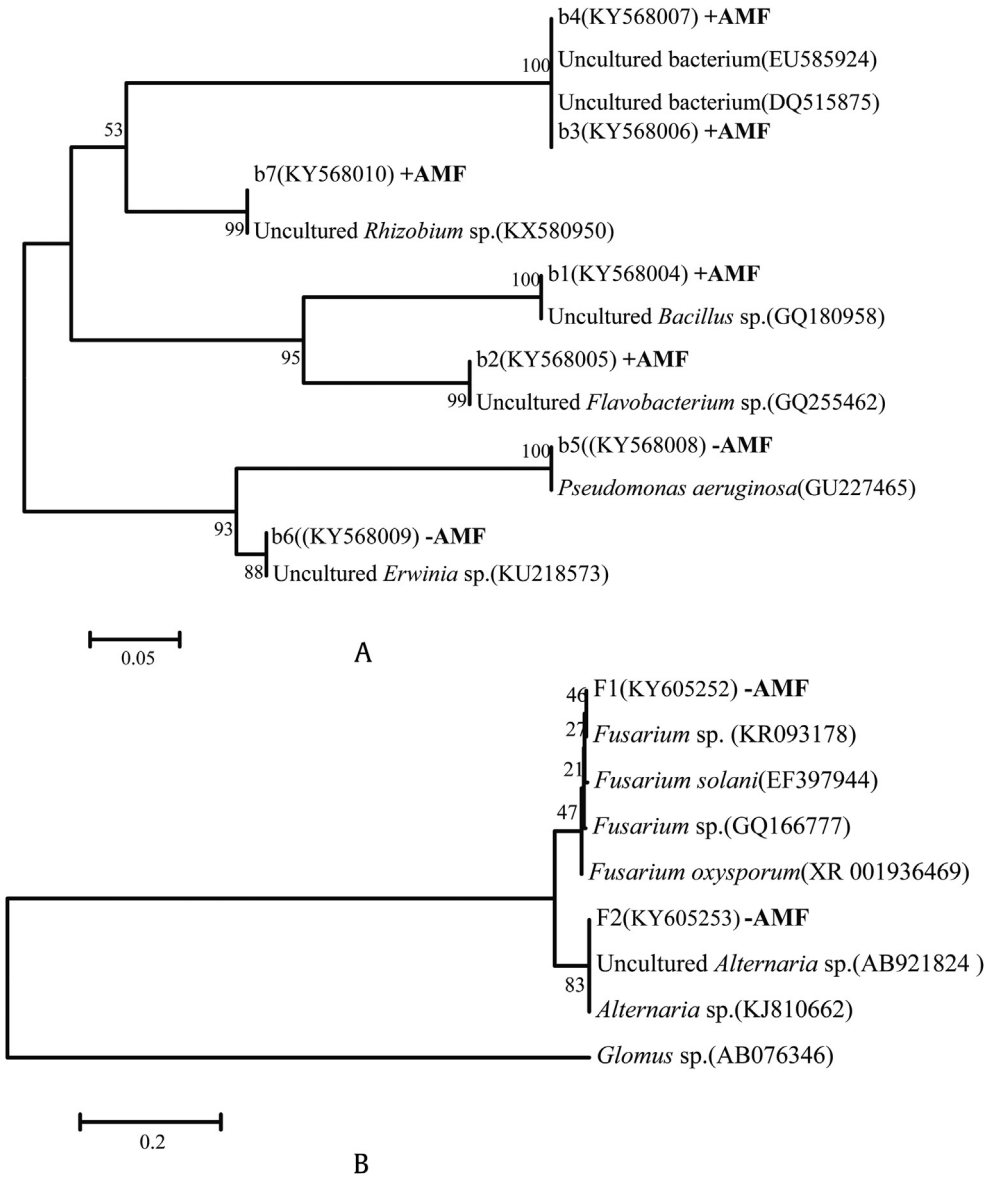

Fig. 6. Analysis of phylogenetic trees for specific bacterial and fungal species based on DGGE bands. (A) Phylogenetic tree of bacterial-specific species of ginseng rhizospheric soil in inoculated (+AMF) and non-inoculated (−AMF) groups. The number at each branch points is the percentage supported by bootstrap in 1000. Bar, 0.05 substitutions per nucleotide position. Numbers in parentheses were the sequence accession number in GenBank. (B) Phylogenetic tree of fungal-specific species of ginseng rhizospheric soil in +AMF and −AMF groups. The number at each branch points is the percentage supported by bootstrap in 1000. Bar, 0.2 substitutions per nucleotide position. Numbers in parentheses were the sequence accession number in GenBank. AMF, arbuscular mycorrhizal fungi; DGGE, denaturing gradient gel electrophoresis.

with those of Sayeed and Zakia's study. It may also indicate that inoculation with AMF can inhibit the emergence of fungal pathogens such as *Fusarium* sp. and *Alternaria* sp. Our research is also in agreement with Kjøller and Rosendahl's research, indicating that plants pre-inoculated with *G. intraradices* were more tolerant to infection with *Aphanomyces euteiches* than non-mycorrhizal plants [45].

5. Conclusion

In the present study, we found that *G. intraradices* can improve the colonization rate in lateral roots of ginseng, increase the level of ginsenoside monomers and total ginsenoside, and improve root activity, polyphenol oxidase activity, and catalase activity. Inoculated *G. intraradices* can change the rhizospheric microbial community. Inoculated *G. intraradices* increased the abundance and bacterial Shannon diversity and decreased the abundance and fungal Shannon diversity. Furthermore, inoculated *G. intraradices* may enhance some plant growth-promoting bacteria and decreased some plant pathogens.

Conflicts of interest

The authors declare that they have no competing interests.

Acknowledgments

This work is financially supported by the Chinese Academic project B (XDB15030103), National Project (2016YFC0501202), the National Natural Science Foundation of China (41571255, 31370144), Key Laboratory Foundation of Mollisols Agroecology (2016ZKHT-05), and 135 Project of Northeast Institute of Geography and Agroecology (Y6H2043001).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jgr.2017.08.005.

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