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An endophytic bacterium isolated from *Panax ginseng* C.A. Meyer enhances growth, reduces morbidity, and stimulates ginsenoside biosynthesis

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ABSTRACT

Ginseng (*Panax ginseng* C.A. Meyer) is known for its therapeutically useful ginsenosides that have anticancer and other pharmacological effects. However, its low levels in plants and the high costs of chemical synthesis make ginsenosides commercially non-viable; as such, strategies for increasing ginsenoside yield are of great interest. The present study reports the isolation of eight novel endophytic bacteria from ginseng leaves, the highest ginsenoside concentration of microbial transformed strain was identified as *Paenibacillus polymyxa*. Inoculation of ginseng plants with *P. polymyxa* by foliar application combined with irrigation enhanced plant growth parameters, reduced morbidity, and increased plant concentration of the ginsenosides (Rg_1, Re, Rf, Rb_1, Rg_2, Rb_2, Rb_3, and Rd) in field experiments. These results indicate that *P. polymyxa* isolated from ginseng is a beneficial endophytic bacterium with biocontrol properties that can enhance the yield and quality of this medicinal plant.

1. Introduction

Endophytes occur in all known plant species and contribute to their hosts by producing substances that provide protection and ultimately increase plant survival (Yu et al., 2010), without showing any signs of infection or producing other negative effects (Araujo et al., 2002; Sturz et al., 2000). As such, endophytes are agents that can potentially be exploited for medicinal, agricultural, and industrial purposes (Brader et al., 2014; Hardoim et al., 2008; Sturz et al., 2000). Beneficial effects for plants include promoting growth (Vendan et al., 2010); providing or mobilizing nutrients such as nitrogen and phosphorus; producing growth regulators such as auxins, gibberellins, and cytokinins; and protecting plants from pathogens through endophyte-mediated de novo synthesis of compounds and antifungal metabolites (Hardoim et al., 2008; Joshee et al., 2007). It has been suggested that microbial isolates from rhizospheric soil can be used to improve secondary metabolite content in plants (Hallmann et al., 1997; Saraf et al., 2014; Zahir et al., 2003) and that endophytes can specifically induce medicinal plants to produce large amounts of secondary metabolites. However, only a fraction of plant species worldwide has been investigated for the presence of plant growth-promoting endophytes (Tiwari et al., 2013).

Ginseng (*Panax ginseng* C.A. Meyer) is an important medicinal plant known for its ginsenosides that have therapeutic, pharmacological, physiological, and ecological benefits. According to traditional Chinese medicine theory, ginseng reinforces qi, promotes blood circulation, improves spleen and lung function, and anchors the mind, and has traditionally been used as a tonic and panacea that promotes longevity (Leung and Wong, 2010; The State Pharmacopoeia Commission of the People's Republic of China, 2010). Most ginsenoside monomers are present at low levels, especially the rare ginseng saponin monomers that have high value and are known to be bioactive in the prevention and treatment of cardiovascular and cerebrovascular diseases, hepatoprotection, immune regulation, and suppressing carcinogenesis (Ackloo et al., 2000; Jang et al., 2008; Lu et al., 2009). The environmental requirements of ginseng are greater than those of most other medicinal plants and, as such, morbidity is one of the main factors affecting both yield and quality. Strategies to increase the growth capacity and content of active ingredients in ginseng are thus the subject of intensive investigation.

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Despite attempts to chemically synthesize ginsenosides, the plant is still the prime source of these agents, but low endogenous levels make them commercially non-viable. Microbial is one way to improve ginsenoside concentration and yield (Dong et al., 2003). Bacterial endophytes isolated from soil are able to change the concentration of ginsenosides Rb_1, Rb_2, and Rc (Itiro et al., 1972); *Aspergillus niger*, *Aspergillus usamii*, *Lactobacillus delbrueckii*, and *Leuconostoc paramesenteroides* produced Rh_2 from Rb_1 via Rd and F2 (Chi and Ji, 2005), and *Paecilomyces bainier* 229-7 transformed Rb_1 into Rd (Ye et al., 2010). Reaction conditions for microbial transformation are simple, relatively low-cost, have strong specificity, and generate very few byproducts; thus, in contrast to other methods such as acid-catalyzed reactions (Shibata et al., 1966), it can be widely applied to enhance scarce, pharmacologically active components.

This study investigated whether an isolated ginseng bacterial endophyte can enhance total ginsenoside concentration in the plant via microbial transformation. In addition, inoculation with the endophytic bacterium was tested in field experiments to determine whether the medicinal value of ginseng can be increased through enhanced growth, decreased morbidity, and increased concentration of rare ginsenosides.

2. Materials and methods

2.1. Plant material and reagents

Healthy, fresh, continuously cropping ginseng aged 1–4 years was obtained from the Jilin Agricultural University medicinal topiary. Ginsenoside standards were purchased from Jilin University (Jilin, China). High performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Fisher Scientific (Waltham, MA, USA). Distilled water was purified using a Milli-Q system (Millipore, Milford, MA, USA).

2.2. Isolation of bacterial endophytes

Endophytes were isolated from healthy and asymptomatic leaves of field-grown ginseng plants. The protocol of surface-sterilized is as follows: leaves were washed thoroughly under tap water for 10 min and dipped in 5% sodium hypochlorite for 5 min, then rinsed four times with sterile water. After soaking in 75% alcohol for 30 s and rinsing four times with sterile water, a sterile filter paper was used to dry the surface of each leaf. To confirm that the surface disinfection process was successful, 200 μL aliquot of the last sterile water was inoculated in solid potato dextrose agar (PDA) medium and cultured at 28 °C for 48–72 h. The leaf tissues were then macerated using a sterile mortar and pestle in a small volume of sterile phosphate buffered saline (PBS, pH 7.4), with sterile quartz sand being added to improve the wall disruption. Samples (100 μL) of tissue extracts were plated onto PDA medium and then stored at 28 °C for 3 days. Colonies of varying morphology were picked and repeatedly re-streaked on PDA medium until the colony morphology of each isolate reached homogenous. A total of eight endophytic bacteria were isolated and purified.

2.3. Microbial inoculation screening for endophytic bacteria

Endophytes were screened as follows: 250-ml triangular flasks with 60 ml water and 3 g of 4-year-old ginseng root powder (sifted through a mesh with a 20–60 μm pore size) were autoclaved together at 121 °C for 20 min; then, they were refrigerated until the solution reached room temperature. A total of 1 ml of the eight endophyte samples (OD_{600} = 0.5) was transferred by pipetting to each of four flasks; four additional flasks were filled with 1 ml deionized water as blank control. All flasks were cultured at 28 °C with shaking for 12 days and then dried in a 50–60 °C water bath. Strains, ginseng powder, autoclaved ginseng powder (blank control) and microbial inoculated ginseng powder samples were extracted using a modified ultrasound-assisted method (Lee et al., 2011; Shi et al., 2010), and a vanillin/perchloric acid method was used to analyze total ginsenoside concentration (Cao et al., 2011). Strain G, which exhibited a marked increase in total ginsenoside concentration, was selected for further study.

2.4. 16S rDNA gene sequence analysis

Endophytic bacteria were isolated from cultures grown overnight, and bacterial genomic DNA was extracted and purified using a previously published method (Mamlouk et al., 2011) with some modifications. The quality and quantity of isolated DNA were verified by agarose gel electrophoresis. The universal forward and reverse primers 27f (5′-GAG AGT TTG ATC CTG GCT CAG-3′) and 1492r (5′-ACG GCT ACC TTG TTA CGA CT-3′) were used for the amplification of the *16S rDNA* gene in strain G. A total of 25 ng bacterial genomic DNA and 5 pmol of each primer were used in the reaction, which was carried out in a thermocycler programmed as follows: 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min; and 72 °C for 10 min. The amplicon was purified with a PCR Cleanup Kit (Axygen Scientific, Inc., Union City, CA, USA) and sequenced by Sangong Biotech (Shanghai, China). Sequence analysis was performed with the Basic Local Alignment Search Tool (BLAST; National Center for Biotechnology Information [NCBI], Bethesda, MD, USA).

2.5. Determination of growth parameters and morbidity of ginseng inoculated with endophytic bacteria

Field experiments were carried out at Jilin Agricultural University from 2011 to 2013. The average weights of 1-, 2-, 3-, and 4-year-old ginseng were 0.5 ± 0.02 g, 1.3 ± 0.05 g, 3.9 ± 0.1 g, and 17.6 ± 0.3 g, respectively. Plants were grown under the same environmental conditions and the planting distance was 10 cm. Bacteria were grown in PDA liquid medium for 48 h on an orbital shaker (160 rpm at 28 °C), and cells were harvested by centrifugation at 3000 rpm for 10 min at 4 °C; pellets were resuspended in sterile saline water (0.8%) at about 10^8 colony-forming units (CFU)/ml. The concentrations used for inoculation were as previously described (Timmusk et al., 2005; Yang et al., 2013).

Ginseng plants, aged 1, 2, 3, and 4 years old, were divided into control (A1′–A3′) and treatment (A1–A3) groups. The controls were treated with distilled water, while the treatment groups were inoculated by A1, foliar application (~10^8 CFU/ml at 50 ml/m^2 sprayed over the entire plant); A2, irrigation (~10^8 CFU/ml at 50 ml/m^2); or A3, a combination of both (~10^8 CFU/ml at 25 ml/m^2 for foliar application and irrigation), for a total of 24 groups (Yang et al., 2013). The field experiment took 1 month every year and was repeated three times over 3 years. During spraying, the soil surface and other plants were covered with plastic to prevent contamination. Growth parameters and morbidity for each plant were recorded upon harvest. Diseased ginseng plants refer to the leaf rust rot and root antiflow rot. The morbidity rate was calculated with the following formula:

$$\text{Morbidity rate} = \frac{\text{total number of diseased plants}}{\text{total number of tested plants}} \times 100.$$

2.6. Ginsenoside extraction and characterization

Ginsenosides were extracted using a method (described in Section 2.3) that has been published elsewhere (Gao et al., 2012; Zang et al., 2011; Zhang et al., 2013). The ginsenoside standards

Table 1
Ginsenoside concentration of different treatment.

	Bacterial strains							
	A	B	C	D	E	F	G	H
Strain	0	0	0	0	0	0	0	0
Ginseng powder	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}	36.10 ± 1.35^{b}
Autoclaved ginseng powder	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}	31.88 ± 0.52^{a}
Ginseng powder/microbial inoculation	30.13 ± 1.08^{a}	29.88 ± 0.38^{a}	32.76 ± 0.98^{a}	34.72 ± 0.83^{b}	36.13 ± 1.15^{b}	34.90 ± 0.96^{b}	56.48 ± 1.35^{c}	37.12 ± 0.53^{b}

Data are means ± standard error ($n = 5$). Numbers followed by the same letter within a column indicate that the values do not differ significantly between treatments at $P < 0.05$ (Duncan's test). Concentration of ginsenoside (mg (kg DW)$^{-1}$). DW: dry weight.

(Rg_1, Re, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3, and Rd) were identified by HPLC using a reverse-phase C18 column (pinnacle, 250 mm × 4.6 mm; inner diameter, 5 μm) (Restek, Bellefonte, PA, USA). The sample injection volume was 20 μL and the column temperature was maintained at 30 °C. The binary elution solvent consisted of acetonitrile (AN) and water (W), and a gradient elution procedure was used as follows: 0–24 min, 18% AN, 82% W; 24–26 min, 22% AN, 78% W; 26–30 min, 26% AN, 74% W; 30–50 min, 32% AN, 68% W; 50–55 min, 33.5% AN, 66.5% W; and 55–65 min, 38% AN, 62% W. The flow rate was maintained at 1.0 ml/min and the absorbance was measured at a wavelength of 203 nm for ginsenoside detection.

2.7. Statistical analysis

Data were subjected to analysis of variance using SPSS v.19.0 (Chicago, IL, USA). The means were compared using Duncan's test at $P < 0.05$ in order to identify significant differences between treatments.

3. Results

3.1. The microbial inoculation endophytes contain high levels of ginsenoside

Eight bacterial endophytes (A–H) were isolated from ginseng. Changes in total ginsenoside concentration were observed in plants after inoculation. Those treated with strain G has the highest total ginsenoside concentration at 56.48 mg/kg, corresponding to an increase of 24.8 mg/kg over the autoclaved ginseng powder (Table 1). Based on these results, strain G was selected for further analysis.

3.2. Colony morphology characterization of strain G

The convex, white/cream-colored circular colonies of strain G were uniformly distributed when cultured on solid medium (A1 and A1′) and was a gram-positive bacterium (B1 and B1′) as been showed in Fig. 1.

3.3. 16S rDNA gene sequence analysis of endophytic bacterium

The *16S rDNA* gene amplicon obtained for strain G showed 99% identity to the partial sequence of the gene in *Paenibacillus polymyxa* (accession number EU239165.1) (Fig. 2). The *16S rDNA* gene sequence of strain G about 1450 bp length was submitted to GenBank (NCBI).

3.4. Effect of endophytes on ginseng growth and morbidity

Plant growth and morbidity increased as a function of age, as is the case for all perennial medicinal plants (Fig. 4). The maximum increases in height and weight and lowest morbidity were observed in plants treated with *P. polymyxa* by foliar application combined with irrigation at all ages. On average, the heights of 1-, 2-, 3-, and 4-year-old plants were 38.44%, 35.24%, 41.90%, and 24.00% higher, respectively, than those of controls of the same age, while average weights in the treatment group were 31.64%, 58.87%, 46.70%, and 18.61% greater, respectively, than for controls. Similarly, morbidity in *P. polymyxa*-treated 1- to 4-year-old plants was 13.64%, 17.67%, 21.67%, and 27.34% lower, respectively, than in ginseng treated with water.

3.5. Effect of endophytes on ginsenoside concentration in ginseng

The calibration curve and correlation coefficients of nine ginsenosides are shown in Table 2. HPLC was carried to determine the ginsenoside concentration of plants in the control group (B1–B4, corresponding to 1- to 4-year-old plants treated with water) and treatment groups (B1′–B4′, corresponding to 1- to 4-year-old plants treated with *P. polymyxa* by foliar application combined with irrigation) (Fig. 4). The nine ginsenosides examined were well resolved and a baseline separation was achieved within 55 min (Fig. 4).

The total ginsenoside concentration in 1- to 4-year-old ginseng plants treated with *P. polymyxa* were 36.83%, 44.52%, 67.96%, and 79.44% higher, respectively, than in control plants (Table 3). In 4-year-old groups, the concentration of Rc was 54.24% lower than that in controls, that of Rd was 308.01% higher. These results

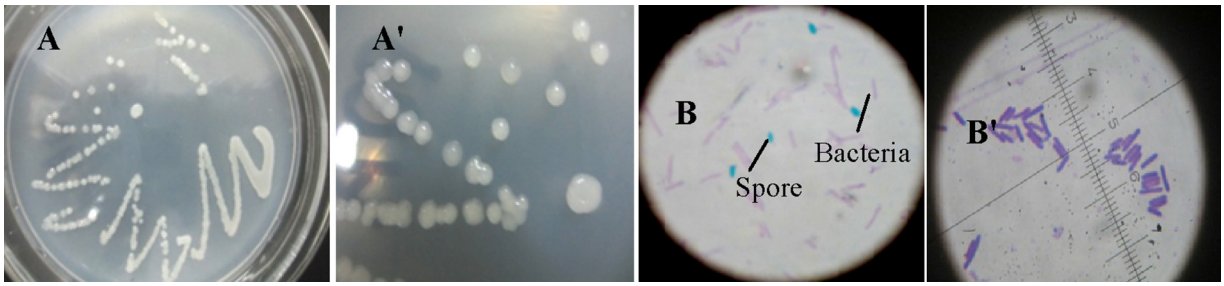

Fig. 1. Colony morphology and Gram staining of bacterial endophytes isolated from *Panax ginseng* C.A. Meyer. (A, A′) Isolates grown on potato dextrose agar formed round whitish colonies. (B, B′) Occasional contaminating spores (*) were observed among bacteria (arrowhead).

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TTGCTGCTTA CCCTTCCCAC CTTGAATGGT ATCATCTAGC GTCATCCGCC CAGTTGCTTC  60
AAGTGAGCTT CGTTTTCGTG CTCATTGAAG GTTACCCTGG CCATTGCCTC GTTTGACAAC 120
GGATATTTCG TTCCCGTATA AAATTGCTTA TCGTTCTGGT TCCAGAACAT CACTGTGAAA 180
TCTTGGTCCG AGCGATTCGC GTTTACAGCT TGGATACCTG ACCGTGCTAC CAGTCTCCCT 240
TCACCTGGCT AGATTCCCTG GAAACAGATA TTGTAAGAAC GCAGGAGAAT CAGGACATGC 300
TTGACATACG GAAGCTGTAT CCGCGAGGGT CTTGTGGAAG AGTGTCTCAG CTGCTCCCAC 360
ACGCTGGAAA CTGGTACCAA GGTGTAGTAT CCTAATTAAA TCTTGATCAA ATTAGGATGC 420
TTTGCCTTGT TACCTGTTAT GCTTTAGAGG GACCAAGTGA TGCAGTCTTG CACAAGACCC 480
TCTTTGATGC GTGTGCCGTA TGTCGATCAT ACTCCGATGC GTGCGCACTA CATATATATG 540
CGTGTCCACT GACTCTCACT CGCTTCACTG ATAACGTGAA CCACGACCGG TATTCCTTGT 600
GTCCACGTGC                                                        610
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Fig. 2. Partial sequence of *Paenibacillus polymyxa* 16 Sr DNA.

indicated that *P. polymyxa* can effectively change the ginsenoside concentration of ginseng.

4. Discussion

The findings presented here demonstrate that endophytic bacteria, specifically *P. polymyxa*, can increase plant growth and ginsenoside concentration and reduced morbidity in ginseng. The variation in ginsenoside concentration in the screened microbial inoculation products indicates that *P. polymyxa* stimulated the synthesis of ginsenosides. Treatment of ginseng with the other seven endophytes also altered total ginsenoside concentration, but the increase relative to control plants was not statistically significant. It was found that the ginsenoside concentration in ginseng powder was reduced by autoclaving, consistent with previous findings (Gui and Ryu, 2014), indicating that ginsenosides are not stable at high temperatures. Many endophytes have been detected in ginseng root (Saraf et al., 2014), with some known to promote plant growth. This is the first report of beneficial endophytes screened by microbial inoculation; in other studies, the screening was done through field experiments that are time-consuming and labor-intensive (Tiwari et al., 2013).

P. polymyxa was isolated for the first time from ginseng (Table 2 and Fig. 3); this species is classified by the Environmental Protection Agency as an organism that has commercial applications as a biocontrol agent and can be used to protect plants from bacterial wilt, lodgepole pine rot (Bent et al., 2002), Verticillium wilt, and rapeseed rot (Beatty and Jensen, 2002). *P. polymyxa* is an alternative to a variety of currently used chemical fungicides. Thus, its functions are twofold: to protect plants against bacterial and fungal soil-borne diseases (Ishii et al., 2000), and increase plant biomass and production. Among 51 endophytes isolated from ginseng, *Bacillus*, *Agrobacterium*, *Pseudomonas*, *Paenibacillus*, and *Stenotrophomonas* were found to enhance plant growth; some of these possessed antifungal activity (Cho et al., 2007; Vendan et al., 2010). The endophyte-mediated enhancement of key active ingredients and growth parameters has been reported in other plants such as *Triticum aestivum*, *Cucumis sativus*, and *Catharanthus roseus* (Larsen et al., 2009; Li et al., 2012; Tiwari et al., 2013). In this study, *P. polymyxa* treatment was associated with increased disease resistance in plants. The effect on growth was most pronounced in groups A1 and A2, with greater increases in height and weight observed in the latter. Indeed, the rank order for effects on ginseng growth and reduction in morbidity was A3 > A2 > A1

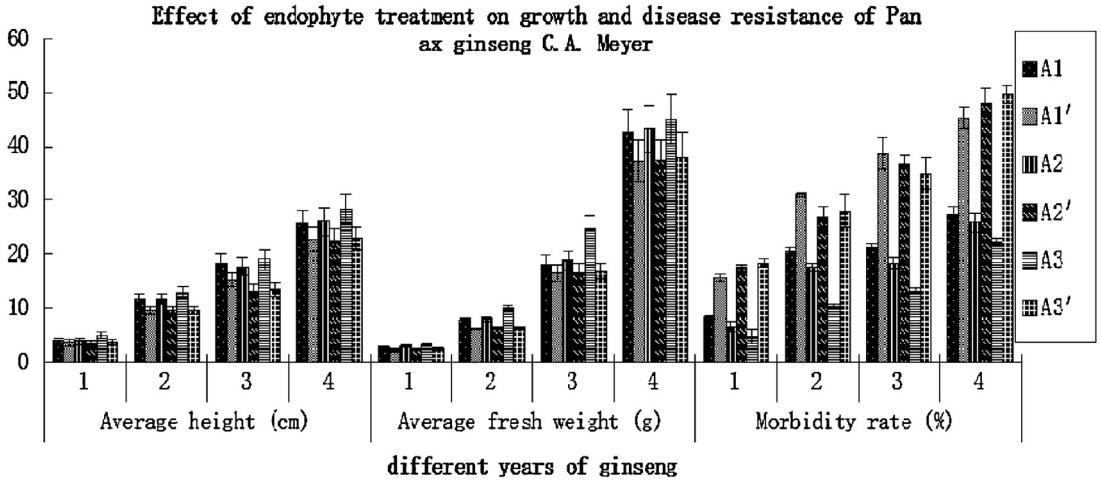

Fig. 3. Effect of endophyte treatment on growth and disease resistance of *Panax ginseng* C.A. Meyer. Plants between the ages of 1 and 4 years were treated with water (A1′–A3′) as a control or were inoculated with *Paenibacillus polymyxa* for A1 (foliar application; ~108/ml CFU at 50 ml/m^2 sprayed over the entire plant); A2, irrigation (~108/ml CFU at 50 ml/m^2); or A3, a combination of both (~108/ml CFU at 25 ml/m^2 for foliar application and for irrigation), for a total of 24 groups. Plant height and weight, as well as disease status (morbidity), were recorded 1 month later.

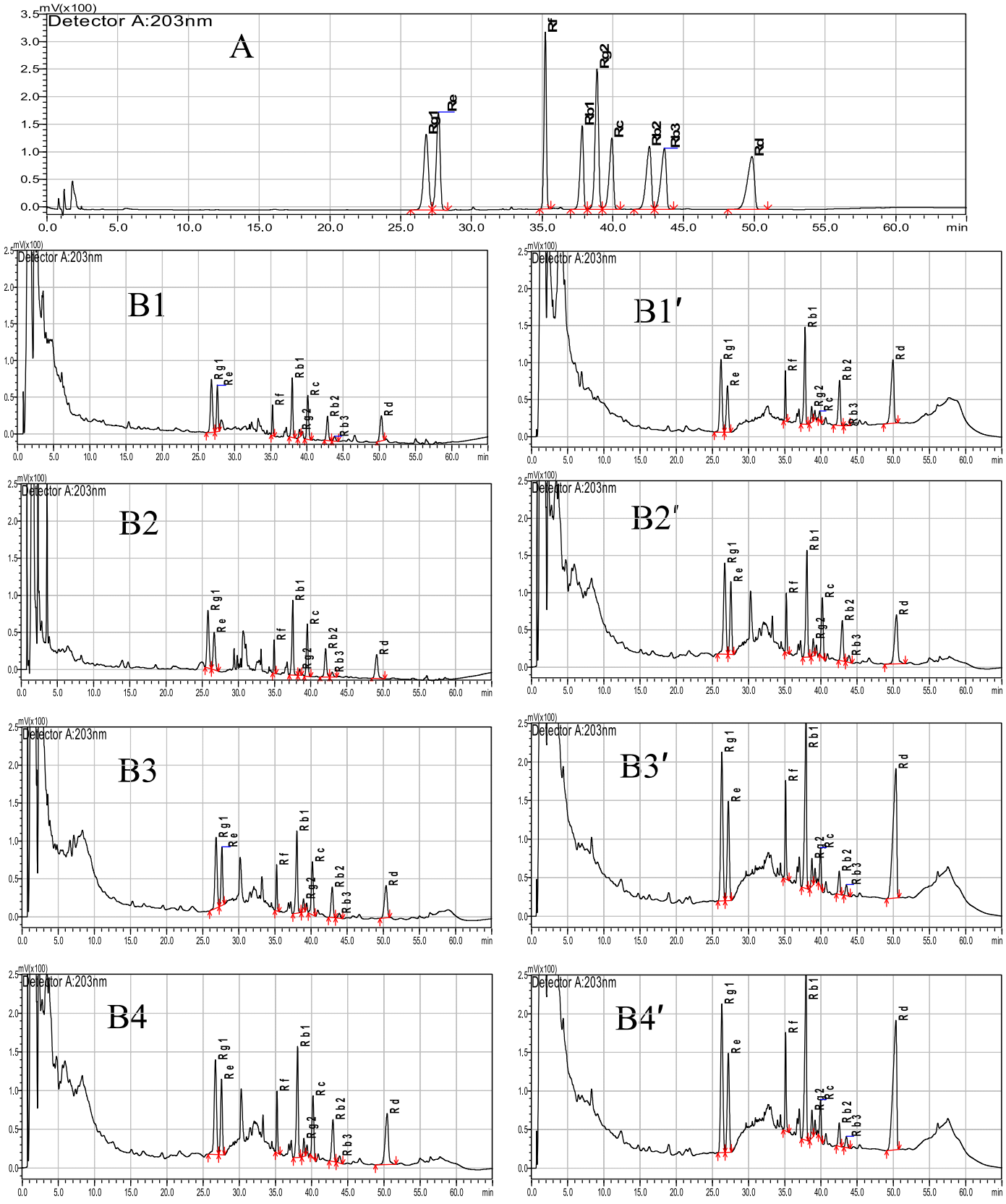

Fig. 4. Determination of ginsenoside concentration in *Panax ginseng* C.A. Meyer by high performance liquid chromatography (HPLC). Plants between the ages of 1 and 4 years were treated with water (B1–B4) as a control, or inoculated with *Paenibacillus polymyxa* by a combination of foliar application and irrigation (~108/ml CFU at 25 ml/m^2 each; B1′–B4′), and the levels of nine ginsenosides (Rg_1, Re, Rf, Rb_1, Rg_2, Rc, Rb_2, Rb_3, and Rd) were detected in each sample. (A) HPLC chromatogram of the combined solution of nine standard ginsenosides.

Table 2
Calibration curves and correlation coefficients of nine ginsenosides.

Ginsenosides	Calibration curve[a]	r
Rg_1	$Y = 1.515611 \times 10^{-7}X - 4.138048 \times 10^{-3}$	0.999989
Re	$Y = 1.511606 \times 10^{-7}X - 3.275162 \times 10^{-3}$	0.999899
Rf	$Y = 1.358943 \times 10^{-7}X - 8.518706 \times 10^{-3}$	0.999902
Rb_1	$Y = 1.936481 \times 10^{-7}X - 4.792064 \times 10^{-3}$	0.999849
Rg_2	$Y = 1.146518 \times 10^{-7}X - 1.797807 \times 10^{-3}$	0.999938
Rc	$Y = 1.823266 \times 10^{-7}X + 3.511306 \times 10^{-3}$	0.999968
Rb_2	$Y = 1.715022 \times 10^{-7}X + 1.706922 \times 10^{-3}$	0.999970
Rb_3	$Y = 1.659856 \times 10^{-7}X + 1.078246 \times 10^{-3}$	0.999845
Rd	$Y = 1.500332 \times 10^{-7}X - 2.796267 \times 10^{-3}$	0.999962

[a] Y and X are the peak area and concentration of the single ginsenoside, respectively.

Table 3
Effect of *Paenibacillus polymyxa* inoculation by a combination of foliar application and irrigation on ginsenoside concentration in *Panax ginseng* C.A. Meyer of different ages.

Age (years)	Ginsenoside[a]									
	Rg_1	Re	Rf	Rb_1	Rg_2	Rc	Rb_2	Rb_3	Rd	Rt
1	C 2.507 ± 0.111	1.515 ± 0.118	0.394 ± 0.051	3.086 ± 0.162	0.115 ± 0.092	2.099 ± 0.170	1.253 ± 0.113	0.206 ± 0.017	0.986 ± 0.091	12.161 ± 1.169
	T 3.065 ± 0.225*	2.202 ± 0.216*	0.951 ± 0.047**	3.779 ± 0.313*	0.270 ± 0.014	0.509 ± 0.040**	2.081 ± 0.179**	0.121 ± 0.010**	3.663 ± 0.201**	16.641 ± 1.243*
2	C 2.701 ± 0.221	1.639 ± 0.124	0.515 ± 0.044	3.231 ± 0.310	0.163 ± 0.009	2.437 ± 0.171	1.370 ± 0.089	0.228 ± 0.021	1.382 ± 0.106	13.666 ± 1.145
	T 3.821 ± 0.114**	2.862 ± 0.079**	1.041 ± 0.056**	4.524 ± 0.334**	0.277 ± 0.016**	1.605 ± 0.079**	2.139 ± 0.107**	0.355 ± 0.032**	3.127 ± 0.206**	19.751 ± 1.789*
3	C 3.051 ± 0.220	1.736 ± 0.079	0.746 ± 0.052	3.418 ± 0.217	0.181 ± 0.011	2.736 ± 0.146	1.511 ± 0.109	0.243 ± 0.017	1.396 ± 0.112	15.018 ± 1.230
	T 4.335 ± 0.247**	3.345 ± 0.148**	1.382 ± 0.109**	5.801 ± 0.308**	0.366 ± 0.024**	1.231 ± 0.106**	1.959 ± 0.179*	0.404 ± 0.031**	6.497 ± 0.417**	25.325 ± 2.232**
4	C 3.105 ± 0.158	2.147 ± 0.216	0.883 ± 0.054	3.689 ± 0.239	0.213 ± 0.017	3.145 ± 0.272	1.774 ± 0.134	0.286 ± 0.015	1.603 ± 0.156	16.845 ± 1.301
	T 5.572 ± 0.365**	3.953 ± 0.247**	1.635 ± 0.131**	6.936 ± 0.512**	0.420 ± 0.033**	1.476 ± 0.104**	1.068 ± 0.105**	0.535 ± 0.047**	8.643 ± 0.688**	30.238 ± 2.253**

C: control groups for ginsenoside concentration in *Panax ginseng* C.A. Meyer of different ages; T: treatment groups for combination of foliar application and irrigation on ginsenoside concentration in *Panax ginseng* C.A. Meyer of different ages.
[a] Concentration of ginsenosides (mg/kg DW). DW: dry weight. Rt: sum of nine ginsenosides ($Rt = Rg_1 + Re + Rf + Rb_1 + Rg_2 + Rc + Rb_2 + Rb_3 + Rd$).
* The significantly different values between treatments and control groups at $P < 0.05$ (Duncan's test).
** The very significantly different values between treatments and control groups at $P < 0.01$ (Duncan's test).

at 10^8 CFU/ml and 50 ml/m^2, in agreement with the findings of others (Yang et al., 2013), suggesting that irrigation may be a more effective method than foliar application for plant inoculation in order to achieve maximum biocontrol. And treatment with *P. polymyxa* by foliar application combined with irrigation could be more effective. The left 7 strains named A–F and H have not been applied in treatment with field ginseng plant. And need further study.

The concentration of eight ginsenosides was increased in ginseng inoculated with *P. polymyxa*. Rc, the only ginsenoside whose level was decreased by treatment (Table 3), was likely consumed in a hydrolytic reaction by ginsenoside glycosidase for the synthesis of other ginsenosides such as Rd. Rc and Rd have been reported to enhance T cell proliferation and marginally increase natural killer cell activity (Berek et al., 2001), and fungi such as the yeasts YS-1, YS-2, *A. niger* HQ-1, and *A. oryzae* MQ-1 can metabolize Rc in the pathway Rc → Mc1 → Mc → CK → PPD (Li et al., 2010). However, to date, there are no reports of *P. polymyxa* transforming ginsenosides in this manner, which is a topic that merits further investigation.

P. polymyxa can be applied to ginseng cultivation to increase harvest and ginsenoside yield. The ginsenoside concentration of microbial inoculation product and field ginseng may have some inner links. The mechanism by which this endophyte induces these effects in ginseng has not yet been linked to a specific factor such as enzymes or methyl jasmonic acid and its derivatives (Cao et al., 2011). The identification of the active ingredient in *P. polymyxa* will be the focus of future studies.

P. polymyxa can be applied to many crops in addition to ginseng as a microbial agent and fertilizer. This approach of using endophytes to reduce morbidity in plants while enhancing their biomass and useful ingredients is in accordance with the so-called green agriculture and represents a sustainable model for harnessing the medicinal potential of a plant. The bacterial endophytes potentially may benefit the production of other types of plants.

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