Research Article

Siderophore-producing rhizobacteria reduce heavy metal-induced oxidative stress in *Panax ginseng* Meyer

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** A B S T R A C T **

**Background:** *Panax ginseng* is one of the most important medicinal plants and is usually harvested after 5 to 6 years of cultivation in Korea. Heavy metal (HM) exposure is a type of abiotic stress that can induce oxidative stress and decrease the quality of the ginseng crop. Siderophore-producing rhizobacteria (SPR) may be capable of bioremediating HM contamination.

**Methods:** Several isolates from ginseng rhizosphere were evaluated by *in vitro* screening of their plant growth-promoting traits and HM resistance. Subsequently, *in planta* (pot tests) and *in vitro* (medium tests) were designed to investigate the SPR ability to reduce oxidative stress and enhance HM resistance in *P. ginseng* inoculated with the SPR candidate.

**Results:** *In vitro* tests revealed that the siderophore-producing *Mesorhizobium panacihumi* DCY119\(^T\) had higher HM resistance than the other tested isolates and was selected as the SPR candidate. *In planta* experiments, 2-year-old ginseng seedlings exposed to 25 mL (500 mM) Fe solution had lower biomass and higher reactive oxygen species level than control seedlings. In contrast, seedlings treated with 10\(^8\) CFU/mL DCY119\(^T\) for 10 minutes had higher biomass and higher levels of antioxidant genes and nonenzymatic antioxidant chemicals than untreated seedlings. When Fe concentration in the medium was increased, DCY119\(^T\) can produce siderophores and scavenge reactive oxygen species to reduce Fe toxicity in addition to providing indole-3-acetic acid to promote seedling growth, thereby conferring inoculated ginseng with HM resistance.

**Conclusions:** It was confirmed that SPR DCY119\(^T\) can potentially be used for bioremediation of HM contamination.

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1. Introduction

*Panax ginseng*, also called Asian or Korean ginseng, is one of the most important and commonly used of the ginsengs [1]. Ginseng is used to maintain body homeostasis and has been found to contain several active compounds including ginsenosides, acid polysaccharides, phenols, and polyethylene compounds that confer many of its pharmacological effects including antioxidative, anticancer, antistress effects, and enhancement of immune system and liver function [2,3]. *P. ginseng* Meyer cultivated in Korea is usually harvested after 4 to 6 years of cultivation; the soil it is grown in is loamy, deep, and well-drained and one of the best habitats for rhizospheric microorganisms [4]. There are a variety of microbes in the ginseng rhizosphere soil that benefit plant growth and ginseng metabolism. Rhizosphere bacteria (rhizobacteria) may influence plants in a direct or indirect manner. For example, rhizobacteria can increase plant growth by supplying nutrients and hormones, producing siderophores to reduce heavy metal (HM) toxicity, and raising induced systemic resistance [5]. Several Korean research groups have isolated novel bacteria strains from the soils in which...
ginseng has been cultivated and screened these strains for their ability to promote plant growth [6,7]. Cultivated ginseng is very vulnerable to abiotic stresses (such as salt, drought, and HM stress) over its long cultivation period, and these stressors can reduce ginseng quality over its 4–6 years of cultivation [8].

HM contamination of soil is an important abiotic stress to plants in addition to being an environmental, ecological, and nutritional hazard. Remediation of HM contamination in soil has been achieved using physical and chemical processes; however, these processes are costly, non-sustainable, and time-consuming [11]. Plant growth-promoting rhizobacteria (PGPR) are plant-associated microorganisms that have been researched for their abilities to bioremediate HM pollution of soils and to promote plant growth in HM-contaminated soils [12]. Among various PGPRs, siderophore-producing rhizobia (SPR) can improve nutrient uptake and promote plant growth under conditions of HM stress.

Our goals in the study were to isolate and characterize several potential novel rhizobacteria as SPR candidates and to investigate if the chosen candidate enhanced the resistance of *P. ginseng* seedlings to HM stress. After identification of a candidate, namely *Mesorhizobium panacihumi* DCY119T, ginseng seedlings were inoculated with or without this candidate and cultivated under Fe stress. Reactive oxygen species (ROS) scavenging activity and antioxidant chemical production by the plants were then assessed. Additionally, microbial antioxidant activity, indole-3-acetic acid (IAA) production, and siderophore production of this strain were assessed.

2. Materials and methods

2.1. Isolation and characterization of isolates

Bacterial strains were isolated from rhizospheric soil samples from fields in which ginseng are cultivated in Gochang-gun (35°26’ 89°N 126°42’ 740°E) and Gyeonggi province (37°14’ 45°5 05° 00.6°E), Republic of Korea. Genomic DNA was extracted and purified using a DNA isolation kit (Gene All Biotechnology, Seoul, Republic of Korea). 16S rRNA gene sequences were amplified by Genotech (Daejeon, Republic of Korea) using the previously described methods [13]. 16S rRNA gene sequences were compiled using Seq-Man software (DNASTAR) and compared with 16S rRNA gene sequences available in the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net).

2.2. In vitro screening of plant growth promotion

Siderophore production was assessed using the chrome azurol S agar assay [14]. Siderophore production was confirmed by a change in color of the halo around colonies in blue–green medium from yellow to red. Phosphate solubilizing ability was verified by the plate screening method developed by Pikovskaya [15]. A clear halo region around the colonies in an opaque medium indicated a positive phosphate solubilization result. Quantitative testing of IAA production was investigated [16] with slight modifications; each strain was cultured by shaking in modified King B broth with and without L-tryptophan (3 mg/mL) after mixing with Salkowski reagent, and optical density (OD) values at 535 nm were read to analyze the production of IAA after 7 days of incubation.

2.3. Determination of heavy metal resistance of the isolated bacterial strains

Strains were grown in Luria Bertani (LB) broth medium supplemented with different metal salts (FeCl3, CdCl2·2.5H2O, HgCl2, CuSO4·5H2O, CoCl2·6H2O, and AlCl3·6H2O) as previously described [17,18]. An 1% (v/v) overnight culture solution inoculated with a single colony was added to 5 mL of LB broth supplemented with different concentrations (25 μM) to 300 μM) of each tested metal salts solution. Strains cultured in the same media without metals served as a control. Metal resistance was evaluated according to growth measured at 600 nm within 0–54 h. If growth was detected during inoculation, the concentration of the selected metal was increased to 50 mM. Inhibitory concentrations were measured using a spectrophotometer at an absorbance of 600 nm against an LB broth (blank) containing the same amount of HM [19]. The resistance of the novel strains isolated from the ginseng rhizosphere was evaluated by calculating the concentration at which there was 50% inhibition of growth (IC50) [20,21].

2.4. Correlation of optical density at 600 nm and colony forming units

*M. panacihumi* DCY119T was incubated in Yeast Mannitol Agar at 30°C for 24 h. After centrifugation at 3000 g for 10 min, precipitated cells were dissolved using sterilized distilled water (SDW) to create serial dilutions with different OD600 values. The different OD values suspensions were smeared on agar plates in triplicate. Colony forming units (CFUs) were counted after incubation at 30°C for 2 days [22].

2.5. Compatibility of strain DCY119T with 2-year-old ginseng seedlings

Based on the correlation between OD600 and CFU/mL determined in Section 2.4, 10^4–10^8 CFU/mL of DCY119T suspensions were prepared. Two-year-old seedlings were first dipped into the different bacterial suspensions for 10 min and then planted in pots (11 cm high and 11 cm diameter) filled with a sterilized soil mixture (vermiculite, perlite, and peat moss at a 3:1:1 ratio to which 25% SDW (v/v) had been added). Seedlings dipped into water without bacteria were the negative control. Each treatment group was replicated in three pots, and each pot contained five seedlings. The temperature was controlled at 25±2°C, and moisture levels were maintained at 60 ± 5%. The photoperiod was adjusted to 16 h of day-time and 8 h of night-time with lamps (Philips TLD-RSFLR22SEX-D 865K) that provided 9500 lux for each covered area within the chambers. Seedlings were watered once weekly with sterilized tap water, which was applied to the bottom of the pots. The symptom development was observed 14 days after inoculation (when leaves of seedlings were opened), sampling and morphological observations were conducted. The biomass (fresh weight [FW] and dry weight [DW]) of each group was also measured for compatibility analysis.
2.6. Assessment of the iron tolerance level of 2-year-old ginseng seedlings

Based on previous reports of the Fe concentration in multi-contaminated soil (139.045 g/kg) and the 0.3–0.8 (3.483–11.098 g/ kg) rusty root index of ginseng rhizosphere soil [23], 25 mL FeCl₃ solution (250 mM, 500 mM, or 1000 mM) was added to each sprouted (after 10 days) seedling pot as same as watering method. For the control, SDW was added instead of the FeCl₃ solution. Cultivation conditions of pots assay are described in Section 2.5. After symptoms appeared, seedlings were collected to record their morphological appearance and for further analysis. Each treatment was replicated in three pots with five seedlings per pot.

2.7. In planta pot tests of resistance of P. ginseng inoculated with DCY119 to Fe stress

2.7.1. DCY119 inoculation and Fe stress treatment

Strain DCY119 was identified as the most promising candidate for enhancing ginseng growth (at concentrations up to 10⁸ CFU/mL) and was therefore selected for P. ginseng inoculation. Seedlings were inoculated with DCY119 as mentioned in Section 2.5. Twenty-five milliliters of 500 mM FeCl₃ solution was added to each pot to induce Fe stress. After symptoms appeared, plants were harvested and analyzed. Four different experimental groups were analyzed: control (non-inoculation and no Fe stress); bacterial treatment (DCY119 inoculation without Fe stress); Fe stress treatment (non-inoculation with Fe stress); and bacteria + Fe stress treatment (DCY119 inoculation with Fe stress). Each treatment was replicated in five pots with five seedlings per pot.

2.7.2. Sampling and morphological observation

On the day of sampling, all seedlings were compared and assigned different grades based on their symptoms, and the disease severity index was then calculated. Some parts of the seedlings were immediately immersed in a liquid nitrogen box and stored at −70 °C in a deep freezer for RNA isolation and H₂O₂ and malondialdehyde (MDA) determination. Other parts of the seedlings were used for morphological observation and measurement of growth parameters such as the FW and DW of the samples and the total phenolic, total flavonoid, total soluble sugar (TSS), and reducing sugar contents. The ginseng samples were first dried at 50 °C to obtain a constant weight for biomass determination.

2.7.3. Quantitative H₂O₂ and MDA measurements

H₂O₂ content was detected using the previous protocol [24] with modification; 0.2 g of fresh tissue was homogenized at 4 °C in 2 mL 0.1% trichloroacetic acid (TCA, w/v). The supernatant was collected after centrifugation at 12,000 g for 5 min at 4 °C. Reaction mixtures (1 mL) comprised 250 µL sample supernatant, 250 µL phosphate buffer (10 mM, pH 7.0), and 500 µL KI (1 M). Absorbance values at 390 nm of each sample were measured. In this study, an H₂O₂ standard curve with a concentration ranging from 0.05 to 0.1 mM was used to calculate H₂O₂ content. Experiments were carried out in triplicate.

MDA was measured to assess lipid peroxidation following the method described in [25] with modification. Briefly, 250 µL of the supernatant homogenized in 0.1% TCA was mixed with 500 µL thiobisbarbituric acid agent (0.5% thiobisbarbituric acid in 20% TCA). The reaction solution was incubated for 30 min in boiling water and cooled rapidly in an ice box. After centrifugation at 10,000 g for 5 min, the absorbance at 532 nm and 600 nm was determined. After subtracting nonspecific absorbance at 600 nm, MDA content in each group was calculated according to the molar extinction coefficient of MDA (155 mM⁻¹ cm⁻¹) [26] and expressed as nmol MDA g⁻¹ FW.

2.7.4. Determination of total phenolic and total flavonoid contents

Total phenolics and total flavonoids in each group were detected using a previously described method [27] with modification. Briefly, 0.5 g of dried material was extracted by 10 mL 80% methanol for 1 h, and this was repeated three times, after which the filtrate was combined for evaporation. The crude extract was redissolved in DW for further compound analysis. For total phenolics analysis, 0.3 mL of extract solution was added to 1.5 mL Folin–Ciocalteu reagent (10 x dilution of 2 N) and allowed to incubate for 5 min after shaking thoroughly. One milliliter of 7.5% Na₂CO₃ solution was added, and the mixture was allowed to sit for 30 min in the dark, and then, the absorbance of the samples was measured at 715 nm. Total phenolic content was calculated from a standard curve using gallic acid as the standard. For total flavonoid measurement, the reaction mixture contained 0.3 mL extract solution, 0.3 mL 5% NaNO₂, and 0.3 mL 10% AlCl₃. After mixing well, the mixture was allowed to sit for 6 min, and then 0.5 mL 1 N NaOH was added. The solution was mixed well, and absorbance was measured immediately at 510 nm. Total flavonoid content was calculated with a calibration curve based on rutin.

2.7.5. Determination of total soluble sugar and reducing sugar contents

The content of TSS in the dried material was detected using Trigoyen’s method [28]. One milliliter of extract solution from Section 2.7.4 was mixed with 5 mL anhydrous reagent and incubated in boiling water. The absorbance value of the mixture was measured at 625 nm. Reducing sugar content was determined following the 3,5-dinitrosalicylic acid (DNS) method [29]. One milliliter of extract solution was mixed with 1 mL DNS reagent. The mixture was incubated in boiling water for 5 min and cooled at room temperature. The absorbance was recorded at 550 nm. TSS and reducing sugars contents were calculated from standard curves prepared using several concentrations of glucose.

2.7.6. RNA extraction and RT-PCR analysis

Total RNA was isolated from frozen samples using TRI Reagent® (Molecular Research Center, Inc., Cincinnati, OH), and 1 µg of isolated RNA from each sample was reverse transcribed with RevertAid™M H Minus M-MuLV Reverse Transcriptase (Fermentas, Waltham, MA) following the manufacturer’s instructions. RT-PCR was performed in a reaction volume of 20 µL consisting of 2 µL of the synthesized cDNA, 10 pmol of forward and reverse primers of each target gene (Supplementary Table S1), 10 µL of Premix Taq™ DNA Polymerase (Takara Bio USA, Inc., Mountain View, CA), and water up to 20 µL using a MyCycler™ thermal cycler (BioRad, Hercules, CA). An initial denaturation step was performed at 95 °C for 5 min, followed by 30–35 cycles of 95 °C for 45 s, 56–62°C for 40 s, and 72°C for 90 s. A final elongation step was performed at 72°C for 5 min. PCR products were electrophoresed through 1.5% agarose gels for visual analysis. Glyceraldehyde 3-phosphate dehydrogenase was used as a control [30].

2.8. In vitro medium assay of the Fe resistance of DCY119

2.8.1. Growth of DCY119 in Fe-medium

Two types of Fe media were assessed: normal culture medium supplemented with different concentrations (0 to 32 mM) of FeCl₃ as described in Section 2.3 and Fe-contaminated soil (see Section 2.7.1) that was extracted using DW. After filter-sterilizing the media through a 0.22 µm filter, a series of soil extract media mixed with the normal medium was prepared (0, 25%, 50%, 75%, 100%). Strain
DCCY119T was inoculated in the different growth media, and OD value at 600 nm was detected and used to express the growth of bacteria after shake culture at 30°C for 48 h.

2.8.2. Production of IAA and siderophores and 2,2-diphenyl-1-picrylhydrazyl scavenging activity of isolated bacterial strains

IAA and siderophore assays were performed as described in Section 2.2. Antioxidant activity was detected using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method of Liu and Pan [31]. The supernatant of each bacteria culture suspension was centrifuged at 10,000 rpm for 10 min, passed through a sterile membrane filter (0.45 μm), and then 0.5 mL of the filtrate was mixed with 1 mL 1 mM DPPH solution and incubated for 20 min at room temperature. The absorbance value of each sample was measured at 517 nm. DPPH solution without sample was used as a control, and ascorbic acid was used as the standard. DPPH free radical scavenging activity of each sample was calculated by the following formula:

\[
\% \text{DPPH scavenging activity} = \left( \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \right) \times 100
\]

3. Results and discussion

3.1. Molecular identification of bacteria

The following five PGPR strains candidate for SPR were isolated from the ginseng rhizosphere: DCY112T, DCY115T, DCY117T, DCY118T, and DCY119T. Based on 16S rRNA gene sequence analysis, the isolates belonged to the following genera: *Rhodanobacter*, *Paraburkholderia*, *Lysobacter*, *Ornithinimicrobium*, and *Mesorhizobium*. Strain information is provided in Supplementary Table S2.

3.2. In vitro assessment of plant growth promotion

The plant growth—promoting activities of the novel strains are shown in Table 1. After 7 days of incubation, strain DCY112T produced 1.5 ± 0.08 μg/mL (without L-tryptophan) and 1.6 ± 0.04 μg/mL (with L-tryptophan) IAA, strain DCY119T produced 4.7 ± 0.74 μg/mL (without L-tryptophan) and 17.6 ± 1.63 μg/mL (with L-tryptophan) IAA. Strains DCY115T, DCY117T, and DCY118T did not produce IAA. Strain DCY119T, which produced IAA when supplied with L-tryptophan, was identified as a potential producer of plant growth regulating hormones. Strains DCY112T, DCY115T, and DCY119T were able to solubilize phosphate as evidenced by a clear halo around the colonies. All novel strains were able to produce siderophores, but strain DCY119T was the strongest producer among the tested strains. Previous studies have demonstrated that the siderophores production activity of strains is directly correlated with enhanced HM resistance and indirectly promotion of plant growth by IAA production ability against HM toxicity [32,33].

3.3. In vitro assessment of heavy metal—resistant bacteria

HM resistance of the bacteria was next determined. Microbial turbidity decreased with an increased concentration of HM. The IC₅₀ values (Fig. 1) of each bacterial strain were calculated [21]. Among all the tested strains, DCY119T was the most resistant to HM in the order of Fe > Al > Cu > Cd > Co > Hg. Given these results, we
Fig. 2. (A) In planta compatibility of DCY119T with 2-year-old *P. ginseng* seedlings. Each group included three pots, each with five seedlings. Bar, 2 cm. (B) Biomass (fresh and dry weights) of *P. ginseng* seedlings inoculated with various concentrations of DCY119T. Error bars indicate the standard deviation (n = 3). Statistical significance using Student *t* test was assigned at *** for *p* < 0.05. FW, fresh weight; DW, dry weight.

Fig. 3. Morphological appearance of *P. ginseng* in response to different Fe stress levels. Each group included three pots with five seedlings per pot. After symptoms appeared, the morphological appearance of ginseng seedlings in each group was assessed. Bar, 2 cm.
selected the siderophore-producing, HM-resistant bacterium DCY119T for further study.

3.4. Compatibility of Mesorhizobium panacihumi DCY119T with *P. ginseng*

By identifying the correlation between OD_{600} and CFU/mL, the required bacterial inoculant amount can be prepared relatively effectively (i.e., there is no need to wait for colony counts on an agar plate). The relationship between OD_{600} values and CFU/mL is shown in Table S3.

After bacterial inoculation for 10–14 days in a pot, the compatibility of DCY119T with *P. ginseng* was assessed by investigating morphological alterations according to the concentration of bacterial suspension (approximately 0, 10^4, 10^6, 10^8, 10^10, 10^12 CFU/mL) as shown in Fig. 2A. No disease symptoms were observed in any group. However, seedlings in the 10^12 CFU/mL treatment showed inhibited growth compared with the control seedlings. After sampling, the biomass of fresh and dry root and shoots were determined (Fig. 2B). DCY119T at 10^8 CFU/mL promoted seedling growth (largest biomass). For this reason, 10^8 CFU/mL of strain DCY119T was selected for further experiments.

3.5. Assessment of the Fe tolerance level of 2-year-old ginseng seedlings

Seedlings were stressed with different concentrations of Fe (0, 250, 500, and 1000 mM) based on previous reports. After symptoms appeared, the morphological appearance of ginseng seedlings in each group was assessed and is shown in Fig. 3. The growth of ginseng seedlings was fully hindered when they were exposed to 1000 mM Fe. Disease symptoms appeared consistent with those seen in the plants suffering from iron toxicity [34,35], and root and shoot growth was inhibited as the previous description that HM stress inhibited growth and decreased the biomass of plants [34,36]. At 500 mM Fe, ginseng seedlings gradually developed

![Fig. 4.](image-url) (A) Morphological observation of *P. ginseng* seedlings inoculated with or without bacteria and then exposed to Fe. Each group included five pots with five seedlings per pot. Bar, 2 cm. (B) Disease severity index values of roots with or without inoculated bacteria then exposed to Fe stress were determined. Bars with the same letters are not significantly different at *p* < 0.05. Scale bar: 2 cm. (C) Root and shoot biomass analysis of *P. ginseng* seedlings inoculated with bacteria and then exposed to Fe. FW, fresh weight; DW, dry weight. Values represent the mean ± SD from three independent experiments. Fe-stress, 500 mM FeCl₃ treatment.

![Fig. 5.](image-url) Estimation of H_2O_2 (A) and MDA (B) contents of each group. Values represent mean ± SD from three independent experiments. Bars with the same letters are not significantly different at *p* < 0.05. (C) Expression of ROS scavenging-related genes and a housekeeping gene (C). CAT, catalase; APX, ascorbate peroxidase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. Fe-stress, 500 mM FeCl₃ treatment; FW, fresh weight; MDA, malondialdehyde; ROS, reactive oxygen species.
yellows on the leaves, and eventually, there was complete wilting of the foliage. Rusty root was also observed, which supports the hypothesis that Fe can induce rusty disease in ginseng roots [23]. We used 500 mM Fe as the HM stress for further study.

3.6. Induction of Fe stress resistance in *P. ginseng* by strain DCY119T

3.6.1. Morphological observations and evaluation of the disease severity index of seedlings under Fe stress

The morphological appearance of seedlings after sampling is shown in Fig. 4A. Fe toxicity directly inhibited ginseng seedling growth, while seedlings inoculated with DCY119T showed significantly reduced Fe stress and grew to a similar extent to control (unstressed) seedlings. To calculate disease severity index (DSI), all roots were graded from 1-6 using the scale described in Supplementary Table S4. DSI was normalized for each isolate using the following equation: 

\[
DSI = \frac{(X_1 \times 1) + (X_2 \times 2) + (X_3 \times 3) + (X_4 \times 4) + (X_5 \times 5) + (X_6 \times 6)}{(X_1 + X_2 + X_3 + X_4 + X_5 + X_6)},
\]

where \(X_1, X_2, X_3, X_4, X_5, \) and \(X_6\) are the number of plants with disease severity scores of 1, 2, 3, 4, 5, and 6, respectively. Based on the described disease symptoms in Table S4, the DSI of each group was calculated and is shown in Fig. 4B. As expected, DCY119T-inoculated groups had some of the lowest DSI values. Several reports have indicated that SPR-like DCY119T can stimulate plant growth, leading to healthy plants and increased plant yields [37,38]. The previous reports indicated that Korean ginseng inoculated with *P. simiae* N3, *B. ginsengiterrae* N11-3, and *C. polytrichastri* N10 were able to support seedlings growth and increase biomass under aluminum stress, which was similar to the results in this study [39]. Stressed seedlings inoculated with DCY119T did not show any symptoms and grew significantly better than the Fe-treated noninoculated group, with no significant difference in growth from the control group. These results demonstrated that DCY119T promotes ginseng growth and protects seedlings against Fe stress.

3.6.2. Determination of the shoot and root biomass of *P. ginseng* seedlings under Fe stress

Shoot and root biomass (FW and DW) are shown in Fig. 4C. Under nonstressful conditions, seedlings inoculated with DCY119T had higher seedling biomass than control seedlings. This result suggests that SPR-DCY119T promotes ginseng growth as a potential PGPR, consistent with the results presented in Section 3.2 and 3.4. HM stress as an abiotic stress can decrease plant yields, which we confirmed in this study. However, strain DCY119T can protect seedlings against Fe toxicity, thereby preventing a decrease in the biomass of ginseng. These results in this study were supported by the published report that plant growth—promoting bacteria as the inoculant of stressed plant could prevent the biomass decrease and improve the plant productivity under stressful environments [40,41].

3.6.3. Effects of H2O2, lipid peroxidation accumulation, and induction of oxidative stress

Various abiotic stressors, especially HM stress, can lead to overaccumulation of ROS (such as hydrogen peroxide, H2O2) in plants. ROS are highly reactive and toxic as they oxidize proteins, lipids, and carbohydrates and damage DNA [42,43]. H2O2 was useful as the secondary messenger in plant signaling networks and could trigger response to various environmental stresses in plants [44]. However, an excessive amount of H2O2 might have activated cell-to-cell signaling as a warning message to other cells, furthermore, high H2O2 accumulation was found in stressed plants and caused oxidative stress. Sukweenadhi et al. [40] reported that excess H2O2 level was found in salt-stressed ginseng seedlings compared with control seedlings, and it could be decreased by antioxidant enzyme expression in bacteria-inoculated stressed seedlings. In this study, H2O2 production was investigated (Fig. 5A). Fe stress increased the H2O2 content of seedlings, indicating that Fe stress induced ROS accumulation and inhibited growth. However, seedlings inoculated with DCY119T exhibited a significant decrease in H2O2 production relative to those not inoculated with this strain, indicating that DCY119T protected the seedlings against ROS accumulation in response to Fe toxicity. Lipid peroxidation is a damaging process that occurs in plants in response to various stresses. The content of MDA, widely used to determine lipid peroxidation level, was also analyzed in this study, and the results are shown in Fig. 5B. Fe-stressed seedlings produced higher amounts of MDA, while inoculation with DCY119T decreased MDA levels in seedlings.

These results demonstrated that Fe stress increased the H2O2 and MDA contents of seedlings and decreased the biomass of the roots and shoots, resulting in growth inhibition of the seedlings. Seedlings inoculated with DCY119T were significantly protected...
against oxidative stress in response to Fe exposure, indicating that DCY119T induced antioxidant defenses in the seedlings.

3.6.4. ROS scavenging antioxidant defenses against Fe stress

To analyze the ROS scavenging activity of seedlings, the expression of two ROS scavenging-related genes, namely \( \text{PgAPX} \) and \( \text{PgCAT} \), was determined, and results are shown in Fig. 5C. These genes were overexpressed in seedlings inoculated with DCY119T compared with noninoculated groups. Higher expression of ROS scavenging-related genes would increase antioxidant activity, thereby enhancing Fe resistance [45].

Nonenzymatic antioxidants react rapidly with free radical intermediates in an autoxidation chain and prevent their progression when added in small amounts to a material. Plants with high levels of antioxidants have been reported to have greater resistance to oxidative damage than those with low levels of antioxidants. Several studies have indicated that the level of oxidative cellular damage in plants exposed to abiotic or biotic stress can be controlled by their antioxidative defense systems. Phenolic and flavonoid compounds are the primary nonenzymatic components of the antioxidant system [42]. Therefore, in addition to enzymatic antioxidant gene expression, we evaluated the level of non-enzymatic antioxidant chemicals (total phenolic and total flavonoid contents); results are shown in Fig. 6A and B. Compared with Fe-stressed seedlings, which had lower phenolic and flavonoid contents than control seedlings, seedlings inoculated with DCY119T had significantly higher total phenolic and flavonoid contents. Therefore, DCY119T helped activate the antioxidant system by increasing the production of nonenzymatic antioxidants, ultimately enhancing seedling resistance to Fe toxicity.

These results suggest that both the increase in expression of enzymatic antioxidant genes and the increase in the production of nonenzymatic antioxidant chemicals induced by DCY119T protected the ginseng seedlings from oxidative stress and enhanced their resistance to Fe.

3.6.5. Estimation of total soluble sugar and reducing sugar contents

Sugars play an important role in plants. Sugar contents of stressed plants can be decreased in response to iron or other HM stresses [34,46]. Several reports also indicated that sugars are key osmolytes that contribute to osmotic adjustment and relate to the stress response [40,47]. Accordingly, TSS and reducing sugar contents of each group seedlings were detected (Fig. 6C and D). These results showed that sugar contents of Fe-stressed seedlings were decreased compared with control seedlings, while seedlings treated with DCY119T showed higher sugar production than stressed seedlings without bacteria to prevent the sugar decrease.
against Fe toxicity, which were indicated again that DCY119\textsuperscript{3} have the HM resistant potential to reduce Fe toxicity in ginseng.

3.7. In vitro medium analysis of the heavy metal resistance of DCY119\textsuperscript{3}

The growth of DCY119\textsuperscript{3} was also evaluated by culturing in culture medium supplemented with different Fe concentrations or different concentrations of extracts from Fe-contaminated soil. OD values at 600 nm were used in this study to express the growth of bacteria (Fig. 7), and DCY119\textsuperscript{3} grew in the culture medium supplemented with Fe and showed the potential Fe resistance. At 16 mM, the growth of DCY119\textsuperscript{3} was significantly starting to decrease; the MIC\textsubscript{Fe} was 32 mM. Meanwhile, the growth of DCY119\textsuperscript{3} was also observed in Fe-contaminated soil extract medium, which indicates that DCY119\textsuperscript{3} can survive in Fe-contaminated soil.

Siderophores production, IAA production activity, and DPPH scavenging activity in the different growth media were evaluated, and results are shown in Figs. 7 and 8. When Fe stress in the culture medium was increased, DCY119\textsuperscript{3} could still produce IAA and siderophores and scavenge DPPH radicals. Similar results were obtained for growth in Fe-contaminated soil extract media. SPR can decrease free metal ions though binding toxic metals with siderophores and reduce oxidative stress, thereby increasing the biomass of plants exposed to HMs [48,49]. IAA produced by SPR can increase plant growth in HM-contaminated soils by inducing root proliferation and promoting nutrient and metal uptake [50].

Together, our results indicate that the siderophore-producing bacterium DCY119\textsuperscript{3} is a potential bioremediating agent that can promote ginseng growth and enhance its HM resistance.

4. Conclusions

Five PGPR-isolated strains as SPR candidates were isolated from the ginseng-rhizospheres of Cochang-gun and Gyeonggi provinces, Republic of Korea. Among the tested bacteria, the siderophore-producing bacterium \textit{M. panachium} DCY119\textsuperscript{3} showed HM resistance, especially to Fe and was selected for further study. Fe-500 mM was sufficient to increase the H\textsubscript{2}O\textsubscript{2} and MDA contents and induce ROS accumulation and oxidative stress in ginseng seedlings. However, preincubation of the seedlings with 10\textsuperscript{8} CFU/mL of DCY119\textsuperscript{3} could directly and indirectly promote ginseng growth against Fe stress. When DCY119\textsuperscript{3}-inoculated seedlings exposed to Fe stress, DCY119\textsuperscript{3} produced siderophores and performed ROS scavenging to directly decrease the toxicity of seedlings and induce ROS scavenging gene overexpression and nonenzymatic antioxidant chemicals, thereby decreasing ROS accumulation and preventing oxidative stress in ginseng seedlings. In addition, DCY119\textsuperscript{3} produced IAA to enhance the nutrient uptake of seedlings, protect them from Fe-induced oxidative stress, and promote plant growth to indirectly reduce Fe stress. In conclusion, strain DCY119\textsuperscript{3} is a siderophore-producing rhizobacterium that shows the potential for bioremediation of HM contamination of ginseng.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.12.008.

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