Short Review

# Bacterial endophytes from ginseng and their biotechnological application

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ABSTRACT

Ginseng has been well-known as a medicinal plant for thousands of years. Bacterial endophytes ubiquitously colonize the inside tissues of ginseng without any disease symptoms. The identification of bacterial endophytes is conducted through either the internal transcribed spacer region combined with ribosomal sequences or metagenomics. Bacterial endophyte communities differ in their diversity and composition profile, depending on the geographical location, cultivation condition, and tissue, age, and species of ginseng. Bacterial endophytes have a significant effect on the growth of ginseng through indole-3-acetic acid (IAA) and siderophore production, phosphate solubilization, and nitrogen fixation. Moreover, bacterial endophytes can protect ginseng by acting as biocontrol agents. Interestingly, bacterial endophytes isolated from *Panax* species have the potential to produce ginsenosides and bioactive metabolites, which can be used in the production of food and medicine. The ability of bacterial endophytes to transform major ginsenosides into minor ginsenosides using β-glucosidase is gaining increasing attention as a promising biotechnology. Recently, metabolic engineering has accelerated the possibilities for potential applications of bacterial endophytes in producing beneficial secondary metabolites.

## 1. Introduction

Ginseng plants, which belong to the *Panax* genus, have been used as an herbal medicine for thousands of years [1]. Ginseng is in the family Araliaceae and is mainly distributed in Asia and North America [2]. More than 20 *Panax* species have been reported worldwide. Five famous *Panax* species, *Panax ginseng* Meyer (Korean ginseng), *P. japonicus* (Japanese ginseng), *P. notoginseng* (Sanchi ginseng), *P. quinquefolius* (American ginseng), and *P. vietnamensis* (Vietnamese ginseng), have been used as an "oriental panacea" for human health [3—6]. Moreover, ginseng has been used in popular functional foods and dietary supplements. While Korea and China are known as the biggest producers and exporters of ginseng in Asia, Canada and the United States are the leading producers in America [7,8]. The size of the global ginseng market was over 620 million USD in 2019. It is expected to increase to 0.9 billion USD by 2027 [2,9].

Ginseng is an herbaceous perennial plant cultivated for its high-valued roots. There are well-known bioactive metabolites (ginsenoside, amino acids, phenolics, alkaloids, polypeptides, and vitamins) found in all parts of ginseng [10,11]. Depending on the processing method, ginseng products can be divided into four types: fresh (fresh product, less than 4 years), white ginseng (dried product, 4—6 years), red ginseng (steamed product, 6 years), and sun ginseng (white ginseng product steamed under high temperature and pressure). Moreover, the different countries have different requirements for ginseng age. For example, in China, 5-6 years old ginseng is required for red ginseng products, but there is no age requirement for fresh ginseng products [1,5,12]. Ginsenosides, which are triterpenoid saponins, are one of the major components of ginseng. More than 150 different ginsenosides have been identified from *Panax* species [8]. Ginsenosides can be classified according to their aglycone skeletons: the dammarane-type and oleanane-type. The dammarane-type includes three subgroups: protopanaxadiol (PPD), protopanaxatriol (PPT), and ocotillol, which can be distinguished by carbohydrate moieties attached to tetracyclic ring at the C-3, C-6, and C-20 positions [13,14]. While *P. ginseng* and *P. notoginseng* are the major sources of

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the PPD and PPT groups, *P. japonicus*, *P. quinquefolius*, and *P. vietnamensis* are the major sources of the ocotillol group. Unlike the dammarane-type, the oleanane-type contains pentacyclic skeletons with aglycon oleanolic acid. Oleanane-type ginsenosides such as $R_O$ and $R_{OA}$ are rare and often undetectable in *P. ginseng* (Fig. 1). According to the presence of sugar moieties in their chemical structures, ginsenosides can be divided into two groups, minor and major ginsenosides. The major ginsenosides are formed through increased glycosylation of dammarane-type aglycones, which are the major components in ginseng. The major ginsenosides include Rb1, Rb2, Rc, Rd, Re, and Rg1. By contrast, the minor ginsenosides are less-glycosylated precursors of major ginsenosides, which are either undetected or present in trace amounts in nature. Several minor ginsenosides are compound K (C—K), Rg2, Rg3, and Rh2 [15]. In addition, the major ginseng latex-like protein 151 (GLP151), lysophosphatidic acid, polysaccharides, oils, polyphenols, and nonprotein nitrogenous substances are important compounds in ginseng [16]. In general, minor ginsenosides are more effective than major ginsenosides in biological and pharmaceutical activities. Ginsenosides have been demonstrated to have numerous pharmaceutical properties (Fig. 1). Ginsenosides have a significant impact on the immune system, diabetes mellitus, and cardiovascular system [17—19]. Ginsenosides have a great potential to treat neurological disorders, including Alzheimer's and Parkinson's disease. Ginseng is also known as a medicinal plant with antianxiety and antidepressant properties [20]. Moreover, ginsenosides have antibacterial, antiviral, antifungal, anti-inflammatory, and antioxidant properties [18,21,22]. Furthermore, ginsenosides are known to enhance sexual endurance and energy [23], and ginsenosides are

used to flavor foods and as a fragrance in the cosmetic industry [24,25].

Endophytes are endosymbionts. They include fungal and bacterial species that colonize tissues of healthy plants without causing any disease [26,27]. The colonization can be intercellular or intracellular. Owing to their ubiquitous presence in all plants, endophytes have been presented as important and promising organisms for study. Endophytes exhibit the ability to produce large bioactive compounds that promote plant growth and control phytopathogens, as well as enhance plant tolerance to biotic and abiotic stresses [28,29]. Moreover, endophytes isolated from medicinal plants can synthesize bioactive compounds. These metabolites can be used as raw materials for medicines, cosmetics, fragrances, and foods [30,31]. In particular, due to the complexity of culture conditions and indiscriminate pesticides, the research of endophytic fungus and their environmental interaction in ginseng population and communities have been challenged attention. The endophytic fungus in field-cultivated ginseng is more characterized than mountain-cultivated ginseng in diversity and biocontrol activity against ginseng pathogens [32,33]. By contrast, bacterial endophytes have much been investigated to identify, characterize, and understand the role of endophytes in ginseng life [34,35]. Furthermore, omics, include genomics, epigenomics, transcriptomics, proteomics, and metabolomics, are rapidly developing to understand the novel bacterial endophytes of ginseng [36,37]. Moreover, fungal and bacterial endophytes are known as good candidates for further researches on their capacity for ginsenosides biosynthesis. While an endophytic bacterium from *P. ginseng* could be transformed the major ginsenosides Rb1 to minor ginsenoside
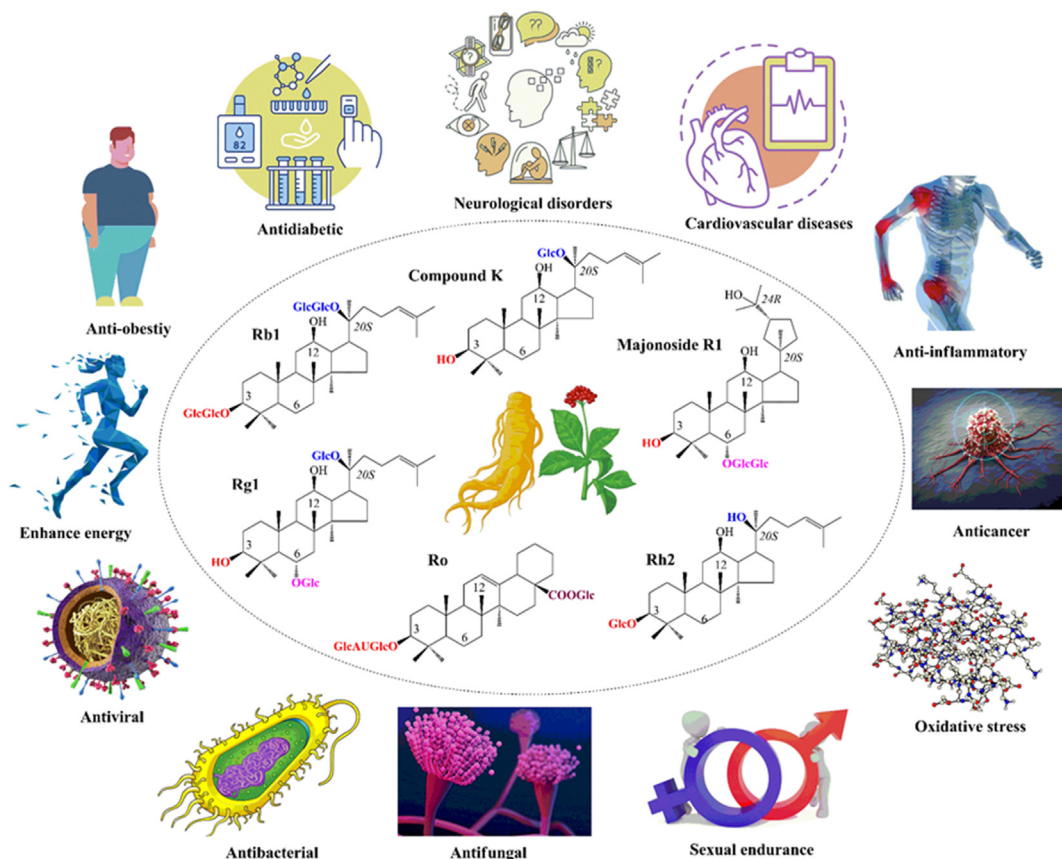


**Fig. 1.** Chemical structures and biological activities of ginsenoside derivatives. Glc, β-D-glucopyranosyl; GlcUA, β-D-gulcuronic acid.
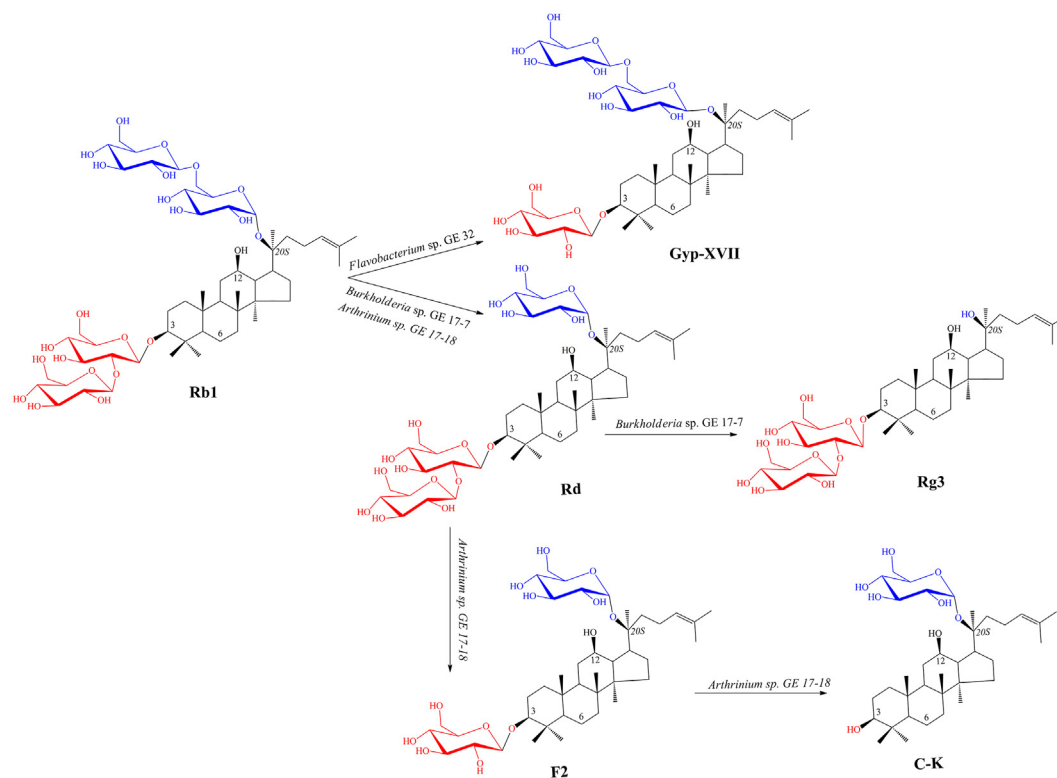
**Fig. 2.** Biotransformation pathway of ginsenoside Rb1 by bacterial and fungal endophytes isolated from *Panax ginseng*.

Rg3, C—K is formed from Rb1 by a fungal endophyte (Fig. 2) [38—40]. In this review, we summarized the current research on bacterial endophytes from *Panax* species and their application in ginsenoside biosynthesis.

## 2. Isolation and diversity of bacterial endophytes from *Panax* species

Several bacterial endophytes have been known to produce compounds that are produced by host plants. Some of these compounds have important effects on treating cancer and diabetes [41—43]. Therefore, the discovery and identification of endophytes from ginseng are necessary to develop novel biologically active compounds for application in the pharmaceutical, cosmetic, and food industries.

### 2.1. Isolation and identification of bacterial endophytes from Panax species

Similar to other endophytes, ginseng endophytes have been isolated and characterized from various tissues and organs of the plant [35,44—47]. Endophytes are traditionally identified using both morphological identification and biochemical characterization [34,48]. In addition, bacterial endophytes can be observed by scanning electron microscopy (SEM) [47]. Furthermore, DNA sequencing is a molecular method of identification. Molecular identification of endophytes can be done by sequencing the internal transcribed spacer (ITS) region or the ribosomal gene (16S or 18S rRNA gene). Noticeably, the 16S rRNA gene is frequently used for the diversity of bacterial endophytes, while the 18S rRNA gene is frequently used for fungal endophytes [33,34,44]. The sequence data were determined by Basic Local Alighment Search Tool (BLAST) analysis. The phylogenetic tree was built *via* the neighbor-joining method in Molecular Evolutionary Genetic Analysis (MEGA) program.

Sequencing of 16S rDNA is the most important approach to identify and construct the phylogenetic tree of bacterial endophytes. Amplification of 16S rDNA is a rapid and easy method for the characterization of large samples [34,45,49]. However, limitations of the 16S rDNA method include short read length and unequal 16S rDNA amplification from closely related species. Moreover, due to sequencing mistakes and complications in their evaluation, operational taxonomic units (OTUs) are another drawback of the 16S rDNA method [50,51]. Therefore, metagenomic sequencing is becoming popular as a powerful tool to replace 16S rDNA sequencing [36]. Metagenomic sequencing not only reduces the potential bias from PCR, it also removes potential contamination of *Panax* genome sequences from the whole genome dataset [52]. Ion Torrent, Illumina, and Pacific Biosciences are common technologies used for detecting bacterial endophytes from *Panax* plants [36,53].

### 2.2. Diversity of bacterial endophytes from Panax plants

Two well-known ginseng species, *P. ginseng* and *P. notoginseng*, are often used to isolate bacterial endophytes [34,45,54]. However, bacterial endophytes isolated from *P. quinquefolius* and *P. vietnamensis* have also been reported in recent years [49,55]. Diverse bacterial endophytes have been isolated from various tissues (roots, stems, leaves, petioles, and seeds) of ginseng plants (Fig. 3). In general, bacterial endophytes from ginseng belong to the phyla Proteobacteria, Actinobacteria, and Firmicutes (Table 1) [45,49,55]. However, the diversity and composition profiles of bacterial endophytes depend on the geographical location, cultivation condition, and plant part, age, and species. For example, 63 bacterial endophytes were isolated from the roots of 5-year-old
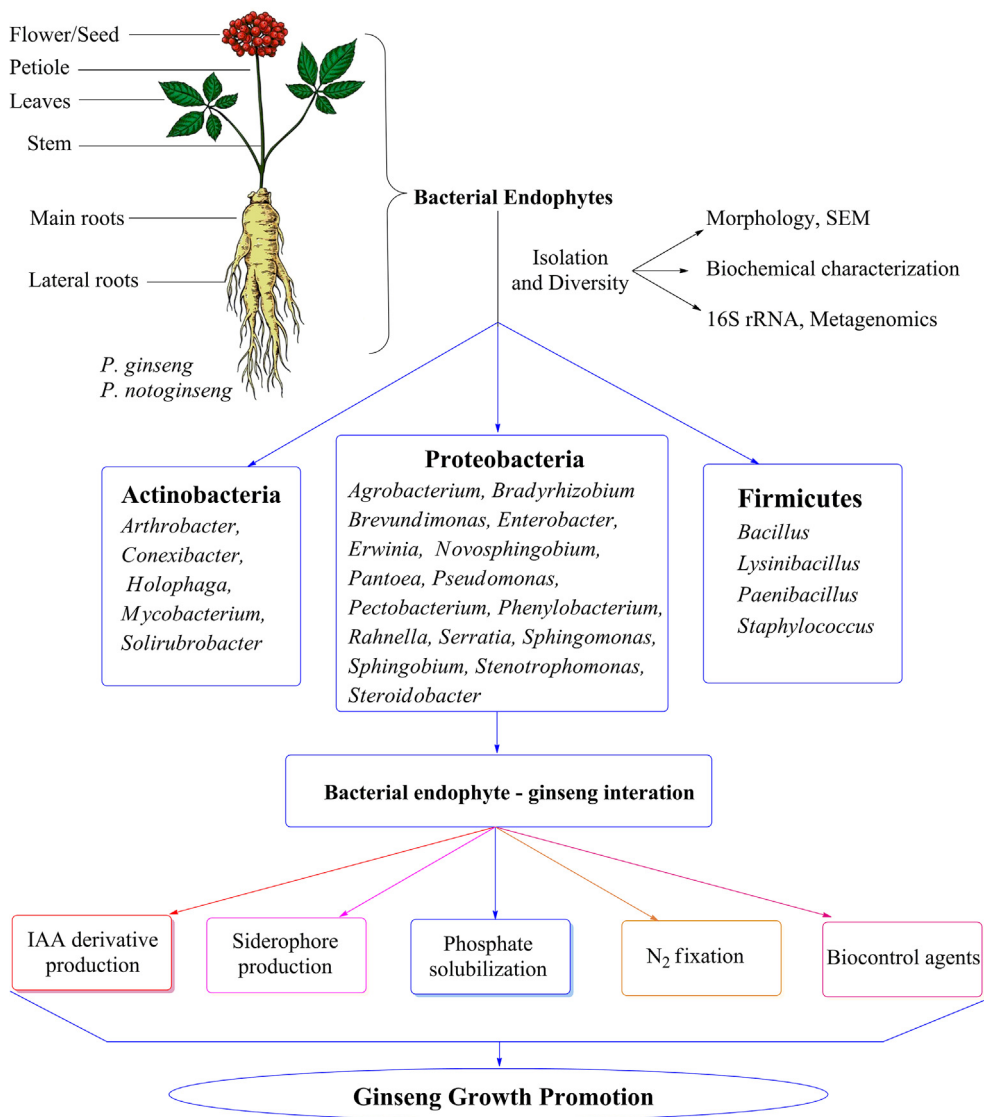
**Fig. 3.** Diversity and plant growth-promoting activity of bacterial endophytes from ginseng.

*P. ginseng* plants at three different sites in Korea [35]. These bacteria were classified into three phyla: Proteobacteria, Actinobacteria, and Firmicutes. *Bacillus* and *Pseudomonas* were identified as dominant endophytes in all three sites. In another study, 1,886 bacterial endophytes were isolated from the roots, stems, and leaves of mountain-cultivated *P. ginseng* at 24 different sites in Korea [34]. Gamma-proteobacteria were the most ubiquitous endophytes across all sites. Among 35 genera of endophytes, seven genera (*Bacillus*, *Burkholderia*, *Paenibacillus*, *Pantoea*, and *Pseudomonas*) were predominant, contributing more than 5% to the total number of endophytes (Table 1). A similar study isolated 137 bacterial endophytes from the seeds of *P. notoginseng* at six sites in China [51]. The *Pseudomonas* genus was the predominant endophyte (Table 1). Growth factors might lead to the variation in the composition and distribution of bacterial endophytes. These growth factors include mean annual rainfall, annual temperature, soil pH, organic matter, phosphate availability, and electrical conductivity. However, the individual climatic and edaphic factors have not yet been demonstrated to affect the diversity of endophytic communities at different geographical locations [34,51].

The diversity of bacterial endophytes of *Panax* plants is associated with plant tissue type [41,45,49,56]. In a 3-year-old *P. notoginseng* plant, the highest diversity of bacterial endophytes was found in the fibril tissue, whereas the leaf tissue had the lowest diversity. The stem and flower tissues had a similar diversity level, following by the root tissue. The bacterial endophyte communities of aboveground parts (flowers, leaves, and stems) and belowground parts (roots and fibrils) were found to vary at the genus level. The genera *Conexibacter*, *Gemmatimonas*, *Holophaga*, *Luteolibacter*, *Methylophilus*, *Prosthecobacter*, and *Solirubrobacter* were considerably less abundant in belowground than in aboveground parts. Conversely, the genera *Bradyrhizobium*, *Novosphingobium*, *Phenylobacterium*, *Sphingobium*, and *Steroidobacter*, were significantly more abundant in belowground parts than in aboveground parts. *Prosthecobacter* was the most abundant bacterial endophyte in *P. notoginseng* at the genus level [45]. Noticeably, *Cyanobacteria* was the most abundant endophyte in the roots of *P. notoginseng*, which might be due to the high fixation of nitrogen in the roots [54]. In mountain-cultivated *P. ginseng*, total species richness of bacterial endophytes was the highest in leaf tissues and the lowest in the stems [34]. Interestingly, stems and roots were found to have

**Table 1**
Diversity of bacterial endophytes from Ginseng plants

| Ginseng Tissues | Age | Total no. of endophytes | Endophytic predominant | Identification method | References |
|---|---|---|---|---|---|
| ***Panax ginseng*** | | | | | |
| Roots | 5 | 63 | *Bacillus*, *Pseudomonas* | 16S rRNA | [35] |
| Stems | 1 | 51 | *Bacillus* | 16S rDNA | [59] |
| | 2 | | *Bacillus*, *Pseudomonas*, *Agrobacterium*, *Stenotrophomonas* | | |
| | 3 | | *Bacillus*, *Microbacterium*, *Agrobacterium*, *Paenibacillus* | | |
| | 4 | | *Staphylococcus* | | |
| Seeds | | 8 | *Caulobacter* sp., *Enterobacter* sp., *Chryseobacterium* sp., *Sphingomonas* sp, *Pseudomonas aeruginosa*, *Duganella violaceusniger*, *Duganella* sp., *Enterobacter asburiae* | 16S rDNA | [63] |
| Leaves | 1-4 | 8 | *Paenibacillus polymyxa* | 16S rDNA | [64] |
| Root, stem, and leaf | 4 | 252 | *Pseudomonas*, *Bacillus*, *Burkholderia*, *Pantoea* and *Paenibacillus* | 16S rDNA | [34] |
| Root, stem, leaf, and flower stalk | 2-6 | 116 | *Acinetobacter*, *Bacillus*, *Microbacterium*, *Pseudomonas*, *Staphylococcus*, *Stenotrophomonas* | 16S rDNA | [56] |
| Roots | 2-6 | 836 | *Actinobacteria*, *Alpha-proteobacteria*, *Gammaproteobacteria*, *Beta-proteobacteria*, *Planctomycetacia*, *Delta-proteobacteria*, *Bacilli*, *Clostridia*, *Acidobacteria*, *Flavobacteria* | Metagenomics rapid annotation | [36] |
| ***Panax notoginseng*** | | | | | |
| Root, stem, petiole, leaf and seed | 3 | 104 | *Bacillus amyloliquefaciens* subsp. *plantarum* and *Bacillus methylotrophicus* | 16S rDNA | [44] |
| Roots | 2-3 | 35 | *Erwinia*, *Stenotrophomonas*, *Pseudomonas*, *Sphingobium* | 16S rDNA | [54] |
| Seeds | 1-3 | 137 | *Pseudomonas*, *Enterobacter*, *Uncultured bacterium*, *Stenotrophomonas* | 16S rDNA and ARDRA | [51] |
| Flower, leaf, stem, root, and fibril | 3 | 46 | *Conexibacter*, *Gemmatimonas*, *Holophaga*, *Luteolibacter*, *Methylophilus*, *Prosthecobacter*, *Solirubrobacter*, *Bradyrhizobium*, *Novosphingobium*, *Phenylobacterium*, *Sphingobium*, *Steroidobacter* | 16S rRNA | [45] |
| ***Panax quinquefolius*** | | | | | |
| Roots | | 16 | *Bacillus*, *Pseudomonas* | 16S rDNA and ITS | [55] |
| ***Panax vietnamensis*** | | | | | |
| Rhizobium | | 24 | *Staphylococcus*, *Enterobacter*, *Serratia*, *Ochrobactrum*, *Arthrobacter* | 16S rDNA | [49] |
| Petioles | | 8 | | | |
| Leaves | | 13 | | | |

similar compositions of bacterial endophytes, while that of leaves were more similar to that of roots than that of stems.

At the phylum level, endophytes belonging to Firmicutes were more abundant in roots and leaves than in stems. Conversely, endophytes belonging to Bacteroidetes were detected only in leaf tissue. In another study, Bacillales was predominant in roots, leaves, and flower stalks. Moreover, Firmicutes, Proteobacteria, and Actinobacteria were abundant in all tissues, except for in flower stalks where Actinobacteria were not found [56]. The differences in bacterial endophytes present in various tissues might be due to the differences in the anatomical structure of each tissue. In addition, it has been postulated that bacterial endophytes can migrate from belowground to aboveground tissues during the development of *Panax* plants [57,58].

*Panax* plants usually grow from the seed to the vegetative and reproductive stages within 1—6 years [11], and the abundance of bacterial endophytes can differ with plant age. For example, the genus *Bacillus* was the dominant strain in the stem of a 1-year-old *P. ginseng* plant [59]. The genera *Bacillus*, *Pseudomonas*, *Agrobacterium*, and *Stenotropphomonas* were dominant in a 2-year-old plant, whereas the dominant genera from a 3-year-old *P. ginseng* plant were *Bacillus*, *Microbacterium*, *Agrobacterium*, and *Paenibacillus*. The *Staphylococcus* genus was dominant in a 4-year-old *P. ginseng* plant. However, Firmicutes was the most abundant at the phylum level and Bacillales at the order level for all ages (2—6 years) [56]. In particular, *B. amyloliquefaciens* and *B. aryabhattai* were present in all ages of *P. ginseng*.

Most recently, a metagenomic analysis indicated that Actinobacteria and Proteobacteria were the dominant phyla in the roots of 2—6-year-old *P. ginseng* plants [36]. This study also found that the most abundant phylum in 3-, 4-, and 5-year-old *P. ginseng* plants was Alpha-proteobacteria, and the highest diversity of bacterial

endophytes was in 3-year-old roots. In addition, diversity of bacterial endophytes from 3-year-old *P. notoginseng* plants was higher than that from 2-year-old plants, even though they were cultivated at the same site [54]. Different nutrient concentrations might cause the differences in endophyte diversity in ginseng with plant age [36,56]. Although there are 17 species in the genus *Panax*, most bacterial endophyte studies were conducted with *P. ginseng* and *P. notoginseng* [60]. The endophyte communities of *P. ginseng* and *P. notoginseng* have been found to differ. Five genera (*Erwina*, *Microbacterium*, *Paenibacillus*, *Pantoea*, *Rahnella*) were found only in *P. ginseng*, while seven genera (*Brevundimonas*, *Churseobacterium*, *Lysinibacillus*, *Myroides*, *Stenotropphomonas*, *Sphingobium*, and *Sphingomonas*) occurred only within *P. notoginseng* [35,36]. The diversity of bacterial endophytes identified from *P. quinquefolius* was similar to that from *P. ginseng*, with *Bacillus* and *Pseudomonas* being the dominant genera [55]. However, *Enterobacter*, *Serratia*, and *Staphylococcus* were identified as dominant bacterial endophytes in *P. vietnamensis* [49]. This might be due to differences in environmental conditions where the two species are grown.

## 3. Associations between bacterial endophytes and ginseng plants

The diverse genera of bacterial endophytes associated with *Panax* plants are responsible for various beneficial biological activities. Bacterial endophytes significantly affect plant growth and protection, antimicrobial activity, biocontrol activity, and novel sources of ginsenoside biosynthesis.

### 3.1. Plant growth-promoting activity

#### 3.1.1. Production of indole-3-acetic acid derivatives

Root growth can be induced by indole-3-acetic acid (IAA). IAA has a significant effect on the uptake of nutrients and minerals *via* the growth of lateral and adventitious roots [61]. Moreover, IAA can improve plant-microbe interactions due to the function of a reciprocal signaling molecule [62]. Many bacterial endophytes isolated from ginseng produce IAA in nutrient broth supplemented with tryptophan as a precursor [59]. These include *Agrobacterium tumefaciens* C58, *Bacillus megaterium* EJH-7, *B. cereus* DS16, *B. subtilis* SC2-4-1, *B. pumilus* CT13, *B. flexus* L2S2, *B. amyloliquefaciens*, *Lysinibacillus sphaericus* C3-41, *L. fusiformis* X-9, *Micrococcus luteus* 164, *Microbacterium phyllosphaerae*, *Paenibacillus glucanolyticus*, *Staphylococcus pasteuri* CV5, and *S. epidermidis* RW35. Among them, *M. luteus* 164 produced the highest amount of IAA. In addition, two bacterial endophytes (*Enterobacter* sp. and *E. asburiae*) isolated from *P. ginseng* seeds produce IAA [63]. Recently, among 252 bacterial endophytes isolated from mountain-cultivated *P. ginseng* in Korea, 166 endophytes (66%) had the ability to synthesize IAA [34]. Proteobacteria and Firmicutes accounted for 75.6% and 19.0% of the bacterial endophytes producing IAA, respectively, followed by Actinobacteria and Bacteroidetes. Interestingly, endophytes can also increase plant growth parameters through foliar application combined with irrigation [64]. In particular, *Paenibacillus polymyxa* isolated from *P. ginseng* was found to induce height and weight growth of *P. ginseng*.

#### 3.1.2. Siderophore production

Siderophores are ferric ion-specific chelating agents, which are secreted under low iron stress [65]. Iron has a significant effect on the growth of *Panax* species because it is an essential element for chlorophyll biosynthesis and chloroplast development [59,66]. Iron deficiency not only leads to chlorosis of leaves but also enhances copper toxicity. Therefore, iron deficiency causes a reduction in plant growth, yield, and quality. Siderophore production by bacterial endophytes may enhance antimicrobial activity [67]. Moreover, the majority of the bacterial endophytes isolated from mountain-cultivated *P. ginseng* in Korea (73.4%) can produce siderophores, with 82% of the siderophore-producing endophytes being from the phylum Proteobacteria and 16% being from Firmicutes [34]. Other siderophore-producing endophytes belong to Actinobacteria and Bacteroidetes. Importantly, the percentage of siderophore producers was the highest in bacterial endophytes isolated from 3-year-old *P. ginseng* plants [36].

#### 3.1.3. Phosphate solubilization and nitrogen fixation

Phosphorus is a major nutrient required for plant growth and promoting N$_2$ fixation [68]. In addition, P has a significant effect on signal transduction, energy transfer, photosynthesis, and respiration. However, P-deficient soils are ubiquitous because P precipitates and fixates with soil constituents [69]. Phosphate-solubilizing endophytes not only reduce the need for chemical fertilizers, but also provide an environmentally friendly and economically feasible alternative strategy, resulting in plant growth improvement [70]. The bacterial endophytes isolated from *P. ginseng* were examined for their ability to solubilize phosphate by detecting extracellular solubilization of tricalcium phosphate with glucose. Several bacterial endophytes showed a high phosphate solubilization rate, including *Lysinibacillus fusiformis*, *B. cereus*, and *B. megaterium* [59]. Moreover, around half (50.0%, 886 out of 1,886) of bacterial endophytes isolated from *P. ginseng* could solubilize phosphate [34]. Noticeably, 96.6% of these isolates belonged to the Proteobacteria.

In addition to IAA and siderophore production and phosphate solubilization, N$_2$ fixation can also stimulate ginseng growth. The partial amplification of the *nifH* gene was used as a method for determining the N$_2$-fixation ability of bacterial endophytes from ginseng. Two bacterial endophytes, *Stenotrophomonas maltophilia* and *A. tumefaciens*, had the *nifH* gene [59]. In another study, five out of eight bacterial endophytes from ginseng seeds could fix N$_2$ on N-free agar medium. These endophytes were *Enterobacter* sp., *E. asburiae*, *P. aeruginosa*, *Duganella* sp., and *D. violaceusniger* [63]. Moreover, three bacterial genes involved in the regulation of nitrogen metabolism (*glnA*, *gltB*, and *nirB*) were identified in the genomes of 2–6-year-old ginseng root tissues [36]. There are three major genes involved in nitrogen metabolism: *glnA* encoding glutamine synthetase, *glnB* encoding nitrogen regulatory PII protein, and *nirB* encoding nitrite reductase [71–73]. Nitrate is transported into the cell by the ABC transporter, and then *nirB* is responsible for reducing nitrate to ammonium. Next, the combination of ammonium and glutamate to form glutamine is catalyzed by *glnA*. The amide nitrogen of glutamine is used as an intermediate to form the various nitrogenous compounds. It was demonstrated that *glnB* has a positive role in the ability of nitrate utilization and a negative role in nitrogen stress response pathways.

### 3.2. Bacterial endophytes as biocontrol agents

Endophytes are known to protect plants from microbial pathogens, weeds, and insects [74,75]. Endophytes can protect plants by disrupting the cell-wall integrity of microbes, through generation and removal of reactive oxygen species (ROS) and by modulating immune system and signaling pathways [76–79]. Bacterial endophytes (*Paneibacillus polyxyma*, *Paneibacillus poae,* and *Bacillus* sp.) isolated from *P. ginseng* exhibit antimicrobial activities against ginseng pathogens (*Rhizoctonia solani*, *Fusarium oxysporum*, *Phythium ultimum*, and *Phytaphthora capsici*) [35]. In addition, three bacterial endophytes isolated from *P. ginseng* (*B. amyloliquefaciens*, *B. megaterium*, *P. frederiskbergenis*, and *Staphylococus saprophyticus*) exhibit antifungal activity against *Cylindrocarpon destructans* and *Botrytis cinerea* [56]. Bacterial endophytes isolated from *P. notoginseng* also showed antagonistic behavior against ginseng pathogens such as *F. oxysporum* and *Ralstonia* sp. and the parasitic nematode *Meloidogyne hapla* [44]. In particular, *Baccillus* spp., *B. amyloliquefaciens* subsp. *plantarum*, and *B. methylotrophicus* were identified as the most dominant species with antagonistic behavior against pathogens in all tissues.

Interestingly, some endophytic groups with antifungal activities can degrade plant cell walls. For example, *P. polymyxa* DSM36, *Bacillus* sp. Bch1, and *Pectobterium crotovora* LMG2466 produce cell wall-degrading enzymes (CWDE; cellulase, xylanase, pectinase, and protease) [44]. The ratio of extracellular activity to cell wall degradation might be critical in determining pathogenicity [80]. Moreover, *Trifolium repens* (white clover) plants possess a cellulase-associated pectinase, which promotes the formation of a nitrogen-fixing nodule of *Lotus*-specific *Rhizobium loti* [81]. In addition, CWDE-producing endophytes might enhance plant-microbe interactions and intercellular colonization in roots [82].

Bacterial endophytes are promising alternatives to chemical agents for controlling ginseng pathogens. Moreover, the ability of bacterial endophytes to high volume fermentation within a short time results in the low-cost production of antimicrobial compounds [83]. Among the 252 bacterial endophytes isolated from mountain-cultivated *P. ginseng* plants, 12 bacteria exhibit an antimicrobial activity against six ginseng pathogens (*Alternaria panax*, *Botrytis cinerea*, *Cylindrocarpon destructans*, *Phytophthora cactorum*, *Pythium* sp., and *Rhizoctonia solani*) [83]. Notably, three endophytes, *Bacillus amyloliquefaciens* EB122, *Burkholderia stabilis* EB159, and

*Pseudomonas koreensis* EB49, showed the highest inhibition rate against the mycelial growth of the six ginseng pathogens tested. *B. stabilis* EB159 had the highest inhibition rate, ranging from 42.3% to 84.7% against all ginseng pathogens. This antimicrobial activity might be due to the production of phenazine, pyrrolnitrin, and other unidentified compounds [84]. Phenazine is a nitrogen-containing heterocyclic compound, which is produced by Eubacteria and some Archaea. Phenazine plays an important role in pathogenesis and competition through ROS generation and cellular redox state modification [85]. Pyrrolnitrin is a tryptophan-derived secondary metabolite with broad antimicrobial activities. Pyrrolnitrin hampers biomolecule synthesis (e.g., DNA, RNA, and protein synthesis), inhibits mitotic cell division, uncouples with oxidative phosphorylation, and hinders some biological mechanisms [86]. Four genes are organized in the *prnABCD* operon for pyrrolnitrin biosynthesis in *B. stabilis* EB159 [53].

*3.3. Ginsenoside induction by bacterial endophytes*

Ginsenosides can occur at various concentrations due to factors affecting the growth and development of ginseng [87,88]. These factors include temperature, humidity, light, water, soil fertility, and *Panax* species. Noticeably, ginsenoside accumulation is also affected by plant age, tissue type, cultivation method, plant pathogen, harvest time, extraction method, and storage process [4,89]. Recently, omics tools not only provide approaches to better understand the growth and ginsenoside biosynthesis in *Panax* plants, but also allow insights into the roles of endophytes during integration with host plants [90,91]. Endophytes exist as elicitors for the accumulation of secondary metabolites in their host plants. For example, the bacterial endophyte *Bacillus altitudinis* acts as an effective elicitor and can improve biomass and ginsenoside accumulation in adventitious root cultures of *P. ginseng* [39]. Furthermore, foliar application and irrigation with *P. polymyxa* increase ginseng growth and ginsenoside accumulation and reduce morbidity in the field [64].

## 4. Bacterial endophytes as novel resources for ginsenoside production

Ginsenosides and related bioactive compounds are uniquely extracted from ginseng plants [3]. However, there is an obvious disadvantage to extracting ginsenosides from ginseng plants. First, ginseng is a slow-growing perennial herb with lower yield. Second, the ginsenoside content of ginseng plants varies and is difficult to control throughout the growth of the plant because it can be affected by weather, nutrients, pathogens, and other conditions [11,92]. Finally, the extraction of ginsenosides from plants is difficult, tedious, and usually yields low amounts. Therefore, mass production of this natural product is limited [93,94]. Interestingly, endophytes can produce the same or similar bioactive compounds as their host plants [27]. There are several hypotheses to explain this trait of endophytes. First, it is believed that endophytes possess a module of genes or full gene clusters responsible for the biosynthesis pathways of plant secondary metabolites. The activation of gene clusters during coexistence and evolution results in the biosynthesis of similar compounds [95]. Second, horizontal gene transfer or genetic recombination can explain the ability of endophytes to produce plant compounds. Horizontal gene transfer allows the transfer of individual genes, gene clusters, or entire chromosomes from host plants into endophytes [96]. The rare ginsenosides such as Rh2 and Rg3 were found to be produced by three bacterial endophytes, which were isolated from *P. ginseng* [97]. Interestingly, strain PDA-2 (*Agrobacterium rhizogenes*) exhibits the ability to produce various ginsenosides such as Rb1 (3.10 mg

L$^{-1}$), F2 (58.20 mg L$^{-1}$), Rh2 (18.60 mg L$^{-1}$), PPD (3.91 mg L$^{-1}$), and Rg3 (62.2 mg L$^{-1}$).

## 5. Bacterial endophytes as a novel platform for biotransformation in minor ginsenoside production

Although ginsenoside-producing endophytes are an eco-friendly and inexpensive alternative to ginseng plants, they often produce low yields. Furthermore, rare ginsenosides not only have higher biological and pharmacological activities but are also more easily absorbed by the human body than major ginsenosides [98,99]. Therefore, it is necessary to develop a process for the mass production of rare ginsenosides.

Due to various advantages of microbial biotransformation over traditional methods and chemical synthesis, biotransformation has received increasing attention as an alternative to convert major ginsenosides into rare ginsenosides. The microbial biotransformation process has limited negative environmental effects because it is carried out at a near neutral pH, ambient temperature, and atmospheric pressure [100]. Importantly, the biotransformation method uses enzyme biocatalysts that are region-specific and have high stereo-selectivity and enantiomer-specific activities, resulting in the production of pure compounds and reducing the need for separation and purification stages [101]. In addition, the biotransformation process results in inexpensive and safe products [102]. Recently, various microbes have been used to produce minor ginsenosides from major ginsenosides. These microbes possess β-glucosidase for hydrolyzing glycosidic bonds of the sugar moieties at the C-3, C-6, and C-20 positions of ginsenosides [103]. Bacterial endophytes isolated from *Panax* plants are some of the most potent strains for biotransformation activities. For example, 38.8% of bacterial endophytes isolated from *P. ginseng* showed the ability to produce β-glucosidase [34]. The β-glucosidase-producing strains belong to Proteobacteria and Firmicutes with 60% and 26%, respectively. Another study found that 27 of the 45 bacterial endophyte strains isolated from *P. vietnamensis* exhibited the ability to hydrolyze the glycosidic bond of Rb [49]. Four active β-glucosidase-producing strains, namely, *Arthrobacter* sp., *Enterobacter* sp., *Ochrobactrump* sp., and *Serratia* sp. have been collected for biotransformation of Rb1 into Rd and Rg3. Interestingly, it was the first time that *Burkholderia* sp. isolated from *P. ginseng* was able to convert major ginsenoside Rb1 to minor ginsenoside Rg3 [38]. *Burkholderia* sp. first hydrolyzes the outer glycosidic linkage at the C-20 position of Rb1 to generate Rd. Then, Rd is continuously converted to Rg3 *via* the hydrolyzation of the inner glycosidic linkage at the C-20 position (Fig. 2). The conversion rate is 98% during optimal fermentation at pH 7.0 and 30 °C for 15 h. Similarly, *Flavobacterium* sp. GE32 isolated from *P. ginseng* showed the ability to convert Rb1 to Rg3 [39]. Furthermore, this strain could also hydrolyze outer glycosidic linkages at the C-3 position to produce Gyp-XVII (Fig. 2). These results indicate that,bacterial endophytes isolated from *Panax* plants have a great potential for application as a novel platform for minor ginsenoside production.

## 6. Conclusion and further perspective

*Panax* plants are one of the most beneficial medicinal plants in the world. Some bacterial endophytes have a great effect on promoting ginseng growth through the production of IAA derivatives and siderophores, phosphate solubilization, and nitrogen fixation. Bacterial endophytes belonging to the phyla Firmicutes, Gammaproteobacteria, and Actinobacteria support ginseng plants for better growth under biotic and abiotic stress. Moreover, bacterial endophytes isolated from *Panax* plants show the capacity to improve the ginsenoside accumulation in their host plants.

Furthermore, bacterial endophytes can produce metabolites with antimicrobial and antifungal activities. Therefore, bacterial endophytes can be used as biocontrol agents. Interestingly, β-glucosidase-producing bacteria have been shown to produce minor ginsenosides through the hydrolyzation of the glycosidic bonds of sugar moieties at the C-3, C-6, and C-20 positions of major ginsenosides. Due to the ability to produce ginsenosides and glucosidase enzymes, bacterial endophytes isolated from *Panax* plants are promising hosts for the production of ginsenosides for applications in foods and medicines.

Although many bacterial endophytes have been identified, these bacteria were mostly isolated from *P. ginseng* and *P. notoginseng*. Therefore, further work is needed to understand the diversity of bacterial endophytes from other *Panax* plants such as *P. vietnamensis*, *P. quinquefolius*, and *P. japonicus*. Next-generation sequencing is driving the discovery of novel endophytes. Whole-genome sequencing not only provides a method to identify isolates accurately but also to understand the interactions between bacterial endophytes and their hosts. Recent omic technologies (metagenomics, transcriptomics, and proteomics) have led to the emergence of the biosynthetic pathways of bioactive secondary metabolites, including ginsenosides, in endophytes. According to our understanding of the biological processes, strategies inducing or repressing genes or gene clusters are necessary to improve the accumulation of ginsenosides in bacterial endophytes. In addition, the development of bacterial endophytes through synthetic biology and metabolic engineering is a promising alternative to enhance the production of ginsenosides and biotransformation products. These properties make bacterial endophytes promising microbial cell factories for ecological and sustainable drug and food production.

## Declaration of competing interest

The authors declare no conflicts of interest.

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## References

[1] Li CP, Li RC. An introductory note to ginseng. Am J Chin Med (Gard City N Y). 1973;1:249—61.

[2] Boopathi V, Subramaniyam S, Mathiyalagan R, Yang DC. Till 2018: a survey of biomolecular sequences in genus *Panax*. J Ginseng Res 2020;44(1):33—43.

[3] Kim DH. Chemical diversity of *Panax ginseng, Panax quinquifolium*, and *Panax notoginseng*. J Ginseng Res 2012;36(1):1—15.

[4] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, et al. Characterization of Korean red ginseng (*Panax ginseng* Meyer): history, preparation method, and chemical composition. J Ginseng Res 2015;39(4):384—91.

[5] Yang W, Qiao X, Li K, Fan J, Bo T, Guo DA, et al. Identification and differentiation of *Panax ginseng, Panax quinquefolium*, and *Panax notoginseng* by monitoring multiple diagnostic chemical markers. Acta Pharm Sin B 2016;6(6):568—75.

[6] Kim K, Nguyen VB, Dong J, Wang Y, Park JY, Lee CL, et al. Evolution of the Araliaceae family inferred from complete chloroplast genomes and 45S nrDNAs of 10 *Panax*-related species. Sci Rep 2017;7:4917.

[7] Baeg IH, So SH. The world ginseng market and the ginseng (Korea). J Ginseng Res 2013;37(1):1—7.

[8] Riaz M, Rahman NU, Zia-Ul-Haq M, Jaffar HZE, Manea R. Ginseng: a dietary supplement as immune-modulator in various diseases. Trends Food Sci Technol 2019;83:12—30.

[9] Future Market Insights. Ginseng market: 2019 analysis and review citrus flavours market by source-wild and cultivated for 2019-2027 [Internet]. 2020 Feb 27. https://www.futuremarketinsights.com/reports/ginseng-market.

[10] Proctor JT, Sullivan AJ, Rupasinghe VP, Jackson CJ. Morphological and ginsenoside differences among North American ginseng leaves. J Ginseng Res 2011;35(2):155—61.

[11] Kim YJ, Zhang D, Yang DC. Biosynthesis and biotechnological production of ginsenosides. Biotechnol Adv 2015;33(6 Pt 1):717—35.

[12] Cho CW, Kim YC, Kang JH, Rhee YK, Choi SY, Kim KT, et al. Characteristic study on the chemical components of Korean curved ginseng products. J Ginseng Res 2013;37(3):349—54.

[13] Shin BK, Kwon SW, Park JH. Chemical diversity of ginseng saponins from *Panax ginseng*. J Ginseng Res 2015;39(4):287—98.

[14] Chu LL, Montecillo J, Bae H. Recent advances in the metabolic engineering of yeasts for ginsenoside biosynthesis. Front Bioeng Biotechnol 2020;8:139.

[15] Biswas T, Mathur AK, Mathur A. A literature update elucidating production of *Panax* ginsenosides with a special focus on strategies enriching the antineoplastic minor ginsenosides in ginseng preparations. Appl Microbiol Biotechnol 2017;101(10):4009—32.

[16] Ratan ZA, Haidere MF, Hong YH, Park SH, Lee JO, Lee JS, et al. Pharmacological potential of ginseng and its major component ginsenosides. J Ginseng Res 2020. https://doi.org/10.1016/j.jgr.2020.02.004.

[17] Kang S, Min H. Ginseng, the 'Immunity Boost': the effects of *Panax ginseng* on immune system. J Ginseng Res 2012;36(4):354—68.

[18] Bai L, Gao J, Wei F, Zhao J, Wang D, Wei J. Therapeutic potential of ginsenosides as an adjuvant treatment for diabetes. Front Pharmacol 2018;9:423.

[19] Kim JH. Pharmacological and medical applications of *Panax ginseng* and ginsenosides: a review for use in cardiovascular diseases. J Ginseng Res 2018;42(3):264—9.

[20] Ong WY, Farooqui T, Koh HL, Farooqui AA, Ling EA. Protective effects of ginseng on neurological disorders. Front Aging Neurosci 2015;7:129.

[21] Wang L, Yang X, Yu X, Yao Y, Ren G. Evaluation of antibacterial and anti-inflammatory activities of less polar ginsenosides produced from polar ginsenosides by heat-transformation. J Agric Food Chem 2013;61(50):12274—82.

[22] Dong W, Farooqui A, Leon AJ, Kelvin DJ. Inhibition of influenza A virus infection by ginsenosides. PLoS One 2017;12(2):e0171936.

[23] Leung KW, Wong AS. Ginseng and male reproductive function. Spermatogenesis 2013;3(3):e26391.

[24] Chen J, Du B, Cai W, Xu B. Ginsenosides and amino acids in flavored ginseng chips as affected by food formulation and processing technology. Lwt-Food Sci Technol 2015;62(1):517—24.

[25] Kim E, Kim D, Yoo S, Hong YH, Han SY, Jeong S, et al. The skin protective effects of compound K, a metabolite of ginsenoside Rb1 from *Panax ginseng*. J Ginseng Res 2018;42(2):218224.

[26] Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 2008;278(1):1—9.

[27] Kusari S, Hertweck C, Spiteller M. Chemical ecology of endophytic fungi: origins of secondary metabolites. Chem Biol 2012;19(7):792—8.

[28] Golinska P, Wypij M, Agarkar G, Rathod D, Dahm H, Rai M. Endophytic actinobacteria of medicinal plants: diversity and bioactivity. Antonie Van Leeuwenhoek 2015;108(2):267—89.

[29] Nalini MS, Prakash HS. Diversity and bioprospecting of actinomycete endophytes from the medicinal plants. Lett Appl Microbiol 2017;64(4):261—70.

[30] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, et al. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 2015;33(8):1582—614.

[31] Gouda S, Das G, Sen SK, Shin HS, Patra JK. Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 2016;7:1538.

[32] Hyde KD, Bussaban B, Paulus B, Crous PW, Lee S, Mckenzie EHC, et al. Diversity of saprobic microfungi. Biodivers Conserv 2007;16:7—35.

[33] Park YH, Kim Y, Mishra RC, Bae H. Fungal endophytes inhabiting mountain-cultivated ginseng (*Panax ginseng* Meyer): diversity and biocontrol activity against ginseng pathogens. Sci Rep 2017;7(1):16221.

[34] Chowdhury MDEK, Jeon J, Rim SO, Park Y-H, Lee SK, Bae H. Composition, diversity and bioactivity of culturable bacterial endophytes in mountain-cultivated ginseng in Korea. Sci Rep 2017;7(1):10098.

[35] Cho KM, Hong SY, Lee SM, Kim YH, Kahng GG, Lim YP, et al. Endophytic bacterial communities in ginseng and their antifungal activity against pathogens. Microb Ecol 2007;54(2):341—51.

[36] Hong CE, Kim JU, Lee JW, Bang KH, Jo IH. Metagenomic analysis of bacterial endophyte community structure and functions in *Panax ginseng* at different ages. 3 Biotech 2019;9(8):300.

[37] Guan Y, Chen M, Ma Y, Du Z, Yuan N, Li Y, Xiao J, Zhang Y. Whole-genome and time-course dual RNA-Seq analyses reveal chronic pathogenicity-related gene dynamics in the ginseng rusty root rot pathogen *Ilyonectria robusta*. Sci Rep 2020;10(1):17122.

[38] Fu Y, Yin ZH, Yin CY. Biotransformation of ginsenoside Rb1 to ginsenoside Rg3 by endophytic bacterium *Burkholderia* sp. GE 17-7 isolated *from Panax ginseng*. J Appl Microbiol 2017;122(6):1579—85.

[39] Fu Y. Biotransformation of ginsenoside Rb1 to Gyp-XVII and minor ginsenoside Rg3 by endophytic bacterium *Flavobacterium* sp. GE 32 isolated from *Panax ginseng*. Lett Appl Microbiol 2019;68(2):134—41.

[40] Fu Y, Yin ZH, Wu LP, Yin CR. Biotransformation of ginsenoside Rb1 to ginsenoside C-K by endophytic fungus *Arthrinium* sp. GE 17-18 isolated from *Panax ginseng*. Lett Appl Microbiol 2016;63(3):196—201.

[41] Shi J, Zeng Q, Liu Y, Pan Z. *Alternaria* sp. MG1, a resveratrol-producing fungus: isolation, identification, and optimal cultivation conditions for resveratrol production. Appl Microbiol Biotechnol 2012;95(2):369–79.

[42] Park YH, Mishra RC, Yoon S, Kim H, Park C, Seo ST, et al. Endophytic *Trichoderma citrinoviride* isolated from mountain-cultivated ginseng (*Panax ginseng*) has great potential as a biocontrol agent against ginseng pathogens. J Ginseng Res 2019;43(3):408–20.

[43] Subramanian M, Marudhamuthu M. Hitherto unknown terpene synthase organization in Taxol-producing endophytic bacteria isolated from marine macroalgae. Curr Microbiol 2020;77(6):918–23.

[44] Ma L, Cao YH, Cheng MH, Huang Y, Mo MH, Wang Y, et al. Phylogenetic diversity of bacterial endophytes of *Panax notoginseng* with antagonistic characteristics towards pathogens of root-rot disease complex. Antonie Van Leeuwenhoek 2013;103(2):299-312.

[45] Dong L, Cheng R, Xiao L, Wei F, Wei G, Xu J, et al. Diversity and composition of bacterial endophytes among plant parts of *Panax notoginseng*. Chin Med 2018;13:41. 2018.

[46] Eevers N, Gielen M, Sanchez-Lopez A, Jaspers S, White JC, Vangronsveld J, et al. Optimization of isolation and cultivation of bacterial endophytes through addition of plant extract to nutrient media. Microb Biotechnol 2015;8(4):707-15.

[47] Song X, Wu H, Yin Z, Lian M, Yin C. Endophytic bacteria isolated from *Panax ginseng* improves ginsenoside accumulation in adventitious ginseng root culture. Molecules 2017;22(6):837.

[48] Singh D, Sharma A, Saini GK. Biochemical and molecular characterisation of the bacterial endophytes from native sugarcane varieties of Himalayan region. 3 Biotech 2013;3(3):205-12.

[49] Tam NT, Tam NP, Nguyen VTH, Hung NK, Huy CN, Ngoc PB, et al. Isolation and screening of endophytic bacteria from Ngoc Linh ginseng (*Panax vietnamensis* Ha et Grushv) for biosynthesis β-glucosidase. J Bio 2018;40(2):154–62. 15625/0866-7160/v40n2.10862.

[50] Poretsky R, Rodriguez -RLM, Luo C, Tsementzi D, Konstantinidis KT. Strengths and limitations of 16S rRNA gene amplicon sequencing in revealing temporal microbial community dynamics. PLoS One 2014;9(4):e93827.

[51] Liu S, Li D, Cui X, Chen L, Nian H. Community analysis of endophytic bacteria from the seeds of the medicinal plant *Panax notoginseng*. J Agric Sci 2017;9(1).

[52] Fadiji AE, Babalola OO. Elucidating mechanisms of endophytes used in plant protection and other bioactivities with multifunctional prospects. Front Bioeng Biotechnol 2020;8:467.

[53] Kim H, Mohanta TK, Park YH, Park SC, Shanmugam G, Park JS, et al. Complete genome sequence of the mountain-cultivated ginseng endophyte *Burkholderia stabilis* and its antimicrobial compounds against ginseng root rot disease. Biol Control 2020;140:104126.

[54] Tan Y, Cui Y, Li H, Kuang A, Li X, Wei Y, et al. Diversity and composition of rhizospheric soil and root endogenous bacteria in *Panax notoginseng* during continuous cropping practices. J Basic Microbiol 2017;57(4):337–44.

[55] Liu X, Zhao Z, Li S, Zhang L, Tian Y, Sun F. The community structure and diversity of the endophytes in American ginseng. Wei Sheng Wu Xue Bao 2015;55(3):330–40.

[56] Hong CE, Kim JU, Lee JW, Lee SW, Jo IH. Diversity of bacterial endophytes in *Panax ginseng* and their protective effects against pathogens. 3 Biotech 2018;8(9):397.

[57] Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB. Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 2005;71(11):7271–8.

[58] Park YH, Lee SG, Ahn DJ, Kwon TR, Park SU, Lim HS, et al. Diversity of fungal endophytes in various tissues of *Panax ginseng* Meyer cultivated in Korea. J Ginseng Res 2012;36(2):211–7.

[59] Vendan RT, Yu YJ, Lee SH, Rhee YH. Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. J Microbiol 2010;48(5):559–65.

[60] Zhang H, Abid S, Ahn JC, Mathiyalagan R, Kim YJ, Yang DC, Wang Y. Characteristics of *Panax ginseng* cultivars in Korea and China. Molecules 2020;25(11):2635.

[61] Wang S, Ren X, Huang B, Wang G, Zhou P, An Y. Aluminium-induced reduction of plant growth in alfalfa (*Medicago sativa*) is mediated by interrupting auxin transport and accumulation in roots. Sci Rep 2016;6:30079.

[62] Spaepen S, Vanderleyden J. Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 2011;3(4):a001438.

[63] Um Y, Kim BR, Jeong JJ, Chung CM, Lee Y. Identification of endophytic bacteria in *Panax ginseng* seeds and their potential for plant growth promotion. Korean J Med Crop Sci 2014;22(4):306–12.

[64] Gao Y, Liu Q, Zang P, Li X, Ji Q, He Z, Zhao Y, Yang H, Zhao X, Zhang L. An endophytic bacterium isolated from *Panax ginseng* C.A. Meyer enhances growth, reduces morbidity, and stimulates ginsenoside biosynthesis. Phytochem Lett 2015;11:132–8.

[65] Leventhal GE, Ackermann M, Schiessl KT. Why microbes secrete molecules to modify their environment: the case of iron-chelating siderophores. J R Soc Interface 2019;16(150):20180674.

[66] Ferreira CMH, Vilas-Boas A, Sousa CA, Soares HMVM, Soares EV. Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. AMB Express 2019;9(1):78.

[67] Rungin S, Indananda C, Suttiviriya P, Kruasuwan W, Jaemsaeng R, Thamchaipenet A. Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). Antonie Van Leeuwenhoek 2012;102(3):463–72.

[68] Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2013;2:587.

[69] Yang Z, Yang S, Van Nostrand JD, Zhou J, Fang W, Qi Q, et al. Microbial community and functional gene changes in arctic tundra soils in a microcosm warming experiment. Front Microbiol 2017;8:1741.

[70] Wang J, Li R, Zhang H, Wei G, Li Z. Beneficial bacteria activate nutrients and promote wheat growth under conditions of reduced fertilizer application. BMC Microbiol 2020;20(1):38.

[71] Suzuki I, Horie N, Sugiyama T, Omata T. Identification and characterization of two nitrogen-regulated genes of the cyanobacterium *Synechococcus* sp. strain PCC7942 required for maximum efficiency of nitrogen assimilation. J Bacteriol 1995;177(2):290-6.

[72] Herrero A, Muro-Pastor AM, Flores E. Nitrogen control in cyanobacteria. J Bacteriol 2001;183(2):411-25.

[73] Yurgel SN, Rice J, Mulder M, Kahn ML. GlnB/GlnK PII proteins and regulation of the *Sinorhizobium meliloti* Rm1021 nitrogen stress response and symbiotic function. J Bacteriol 2010;192(10):2473–81.

[74] Ek-Ramos MJ, Gomez-Flores R, Orozco-Flores AA, Rodriguez-Padilla C, Gonzalez-Ochoa G, Tamez-Guerra P. Bioactive products from plant-endophytic gram-positive bacteria. Front. Microbiol 2019;10:463.

[75] Yu H, Zhao J, You J, Li J, Ma H, Chen X. Factors influencing cultivated ginseng (*Panax ginseng* C. A. Meyer) bioactive compounds. PLoS One 2019;14(10):e0223763.

[76] Fischer D, Gessner G, Fill TP, Barnett R, Tron K, Dornblut K, et al. Disruption of membrane integrity by the bacterium-derived antifungal Jagaricin. Antimicrob Agents Chemother 2019;63(9):e00707–19.

[77] Huang H, Ullah F, Zhou DX, Yi M, Zhao Y. Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 2019;10:800.

[78] Vannier N, Agler M, Hacquard S. Microbiota-mediated disease resistance in plants. PLoS Pathog 2019;15(6):e1007740.

[79] Cui JL, Wang YN, Jiao J, Gong Y, Wang JH, Wang ML. Fungal endophyte-induced salidroside and tyrosol biosynthesis combined with signal cross-talk and the mechanism of enzyme gene expression in *Rhodiola crenulata*. Sci Rep 2017;7(1):12540.

[80] Scheffers DJ, Pinho MG. Bacterial cell wall synthesis: new insights from localization studies. Microbiol Mol Biol Rev 2005;69(4):585–607.

[81] Verma SC, Ladha JK, Tripathi AK. Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. J Biotechnol 2001;81:127–41.

[82] Suto M, Takebayashi M, Saito K, Tanaka M, Yokota A, Tomita F. Endophytes as producers of xylanase. J Biosci Bioeng 2002;93:88–90.

[83] Chowdhury MDEK, Bae H. Bacterial endophytes isolated from mountain-cultivated ginseng (*Panax ginseng* Mayer) have biocontrol potential against ginseng pathogens. Biol Control 2018;126:97–108.

[84] Chandler D, Bailey AS, Tatchell GM, Davidson G, Greaves J, Grant WP. The development, regulation and use of biopesticides for integrated pest management. Philos Trans R Soc B 2011;366(1573):1987–98.

[85] Llontop MME, Hurley K, Tian L, Galeano VAB, Wildschutte HK, Marine SC, et al. Exploring rain as source of biological control agents for fire blight on apple. Front Microbiol 2020;11:199.

[86] Pawar S, Chaudhari A, Prabha R, Shukla R, Singh DP. Microbial pyrrolnitrin: natural metabolite with immense practical utility. Biomolecules 2019;9(9):443.

[87] Szakiel A, Paczkowski C, Henry M. Influence of environmental abiotic factors on the content of saponins in plants. Phytochem Rev 2011;10:471–91.

[88] Kim DS, Song M, Kim SH, Jang DS, Kim JB, Ha BK, et al. The improvement of ginsenoside accumulation in *Panax ginseng* as a result of γ-irradiation. J Ginseng Res 2013;37(3):332–40.

[89] Oh JY, Kim YJ, Jang MG, Joo SC, Kwon WS, Kim SY, et al. Investigation of ginsenosides in different tissues after elicitor treatment in *Panax ginseng*. J Ginseng Res 2014;38(4):270–7.

[90] Ma R, Sun L, Chen X, Mei B, Chang G, Wang M, et al. Proteomic analyses provide novel insights into plant growth and ginsenoside biosynthesis in forest cultivated *Panax ginseng* (F. Ginseng). Front Plant Sci 2016;7:1.

[91] Xue L, He Z, Bi X, Xu W, Wei T, Wu S, et al. Transcriptomic profiling reveals MEP pathway contributing to ginsenoside biosynthesis in *Panax ginseng*. BMC Genomics 2019;20(1):383.

[92] Lee HJ, Jeong J, Alves AC, Han ST, In G, Kim EH, et al. Metabolomic understanding of intrinsic physiology in *Panax ginseng* during whole growing seasons. J Ginseng Res 2019;43(4):654–65.

[93] Zhang MM, Wang Y, Ang EL, Zhao H. Engineering microbial hosts for production of bacterial natural products. Nat Prod Rep 2016;33(8):963–87.

[94] Chemat F, Vian MA, Fabiano-Tixier AS, Nutrizio M, Jambrak AR, Munekata PE. A review of sustainable and intensified techniques for extraction of food and natural products. Green Chem 2020;22:2325–53.

[95] Hardoim PR, van Overbeek LS, Berg G, Pirttila AM, Compant S, Campisano A, et al. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 2015;79(3):293–320.

[96] Yue J, Hu X, Sun H, Yang Y, Huang J. Widespread impact of horizontal gene transfer on plant colonization of land. Nat Commun 2012;3:1152.

[97] Yan H, Jin H, Fu Y, Yin Z, Yin C. Production of rare ginsenosides Rg3 and Rh2 by endophytic bacteria from *Panax ginseng*. J Agric Food Chem 2019;67(31): 8493—9.

[98] Geraldi A. Advances in the production of minor ginsenosides using micro-organisms and their enzymes. BIO Integration 2020;1(1):15—24.

[99] Li W, Fan D. Biocatalytic strategies for the production of ginsenosides using glycosidase: current state and perspectives. Appl Microbiol Biotechnol 2020;104:3807—23.

[100] Hegazy ME, Mohamed TA, ElShamy AI, Mohamed AE, Mahalel UA, Reda EH, et al. Microbial biotransformation as a tool for drug development based on natural products from mevalonic acid pathway: a review. J Adv Res 2015;6(1):17—33.

[101] de Carvalho CC. Whole cell biocatalysts: essential workers from nature to the industry. Microb Biotechnol 2017;10(2):250—63.

[102] Hug JJ, Krug D, Muller R. Bacteria as genetically programmable producers of bioactive natural products. Nat Rev Chem 2020;4:172—93.

[103] Yu S, Zhou X, Li F, Xu C, Zheng F, Li J, et al. Microbial transformation of ginsenoside Rb1, Re and Rg1 and its contribution to the improved anti-inflammatory activity of ginseng. Sci Rep 2017;7(1):138.