Short Review

Bacterial endophytes from ginseng and their biotechnological application

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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.

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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 oco-tillol, 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
the PPD and PPT groups, *P. japonicus*, *P. quinquefolius*, and *P. vietnamensis* are the major sources of the ocytillol group. Unlike the dammarane-type, the oleanane-type contains pentacyclic skeletons with aglycon oleanolic acid. Oleanane-type ginsenosides such as Rg and ROA 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 non-protein 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 ginseside.

**Fig. 1.** Chemical structures and biological activities of ginsenoside derivatives. Glc, β-D-glucopyranosyl; GlcUA, β-D-guluronic acid.
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
*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, Phenyllobacterium, 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

![Fig. 3. Diversity and plant growth-promoting activity of bacterial endophytes from ginseng.](image-url)
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 endophyte 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 Panax ginseng plant [59]. The genera Bacillus, Pseudomonas, Agrobacterium, and Stenotrophomonas were dominant in a 2-year-old plant, whereas the dominant genera from a 3-year-old plant were Bacillus, Microbacterium, Agrobacterium, and Paenibacillus. The Staphyloccocus genus was dominant in a 4-year-old Panax 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 (Erwinia, Microbacterium, Paenibacillus, Pantoea, Rahnella) were found only in P. ginseng, while seven genera (Brevundimonas, Chryseobacterium, Duganella, Myroides, Stenotrophomonas, 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.

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**Table 1**

| 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 rRNA | [59] |
| 2 | | | Bacillus, Pseudomonas, Agrobacterium, Stenotrophomonas | |
| 3 | | | Bacillus, Microbacterium, Agrobacterium, Paenibacillus | |
| Seeds | 8 | | Staphylococcus | 16S rRNA | [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**

| Age Total no. of endophytes | Bacillus amyloliquefaciens subsp. plantarum and Bacillus methylotrophicus | 16S rDNA | [44] |
|-----------------------------|------------------------------------------------------------------------|-----------|---|
| Roots, stem, petiole, leaf and seed | 3 | 104 | 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 | [51] |
| Flower, leaf, stem, root, and fibril | 3 | 46 | Conexibacter, Gemmatimonas, Holophaga, Luteolibacter, Methyllophylus, Proshhexobacter, Sphingomonas | 16S rDNA | [45] |

**Panax quinquefolius**

| Age Total no. of endophytes | Bacillus, Pseudomonas | 16S rDNA and ITS | [55] |
|-----------------------------|----------------------|------------------|---|
| Roots | 16 | | |

**Panax vietnamensis**

| Age Total no. of endophytes | Staphylococcus, Enterobacter, Serratia, Ochrobactrum, Arthrobacter | 16S rDNA | [49] |
|-----------------------------|---------------------------------------------------------------|-----------|---|
| Rhizobium | 24 | | |
| Petioles | 8 | | |
| Leaves | 13 | | |
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 CS8, Bacillus megaterium EJH-7, B. cereus DS16, B. subtilis SC2-4-1, B. pumilus CT13, B. flexus L252, B. amyloliquefaciens, Lysinibacillus sphaericus C3-41, L. fusiformis X-9, Micrococcus luteus 164, Microbacterium phyllophthoraeae, Paenibacillus glucanolyticus, Staphylococcus pasteurii 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 [53]. 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 18.0% of the bacterial endophytes producing IAA, respectively. Moreover, Actinobacteria and Bacteroidetes account for 8.5% of the total endophytes producing IAA. Interestingly, IAA 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 Punan 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 N₂ 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, N₂ fixation can also stimulate ginseng growth. The partial amplification of the nirB gene was used as a method for determining the N₂-fixation ability of bacterial endophytes from ginseng. Two bacterial endophytes, Stenotrophomonas maltophilia and A. tumefaciens, had the nirB gene [59]. In another study, five out of eight bacterial endophytes from ginseng seeds could fix N₂ on N-free agar medium. These endophytes were Enterobacter sp., E. asburiae, P. aeruginosa, Duganella sp., and D. violacesgenus [63]. Moreover, three bacterial genes involved in the regulation of nitrogen metabolism (glnA, glnB, 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 PI 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 (Pannebacillus polysyyma, Panebacillus poae, and Bacillus sp.) isolated from P. ginseng exhibit antimicrobial activities against ginseng pathogens (Rhizoctonia solani, Fusarium oxysporum, Phytophthum ultimum, and Phytophthora capsici) [35]. In addition, three bacterial endophytes isolated from P. ginseng (B. amyloliquefaciens, B. megaterium, P. frederikskbergensis, and Staphylococcus 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. polysyyma DSM36, Bacillus sp. Bch1, and Pectobacterium crotonovora LMG24466 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...
three bacterial endophytes, which were isolated from ginsenosides such as Rh2 and Rg3 were found to be produced by chromosomes from host plants into endophytes [96]. The rare allows the transfer of individual genes, gene clusters, or entire gene transfer or genetic recombination can explain the ability of endophytes can produce the same or similar bioactive compounds the biosynthesis of similar compounds [95]. Second, horizontal biosynthesis pathways of plant secondary metabolites. The acti-

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. Notably, 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 (3.30 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 processes [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-glucosi-
dase-producing strains, namely, Arthrobacter sp., Enterobacter sp., Ochrobactrum 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 hy-
drolyze 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, Gam-
maporeteobacteria, 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 Panax ginseng and Panax notoginseng. Therefore, further work is needed to understand the diversity of bacterial endophytes from other Panax plants such as Panax vietnamensis, Panax quinquefolius, and Panax 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 increasing 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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