MINI-REVIEW

Endophytes: the novel sources for plant terpenoid biosynthesis

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Abstract

Terpenoids are natural compounds predominantly present in plants. They have many pharmaceutical and/or nutritional functions, and have been widely applied in medical, food, and cosmetics industries. Recently, terpenoids have been used in the clinical treatment of COVID-19 due to the good antiviral activities. The increasing demand for terpenoids in international markets poses a serious threat to many plant species. For environmentally sustainable development, microbial cell factories have been utilized as the promising platform to produce terpenoids. Nevertheless, the bioproduction of most terpenoids cannot meet commercial requirements due to the low cost-benefit ratio until now. The biosynthetic potential of endophytes has gained attention in recent decades owing to the continual discovery of endophytes capable of synthesizing plant bioactive compounds. Accordingly, endophytes could be alternative sources of terpenoid-producing strains or terpenoid synthetic genes. In this review, we summarized the research progress describing the main and supporting roles of endophytes in terpenoid biosynthesis and biotransformation, and discussed the current problems and challenges which may prevent the further exploitation. This review will improve our understanding of endophyte resources for terpenoid production in industry in the future.

Key points

• *The mechanisms employed by endophytes in terpenoid synthesis in vivo and in vitro.*
• *Endophytes have the commercial potentials in terpenoid bioproduction and biotransformation.*
• *Synthetic biology and multiomics will improve terpenoid bioproduction in engineered cell factories.*

Keywords Endophyte · Terpenoid · Biosynthesis · Biotransformation · Microbial cell factory

Introduction

Terpenoids, also referred to as isoprenoids, are a large family of the most abundant natural products in nature derived from isoprene units. They exist in almost every organism, but are mainly synthesized by plants as secondary metabolites. To date, more than 80,000 terpenoids have been identified, some of which have important medicinal, physiological, metabolic, communication, and defense functions, and are widely utilized in food, cosmetics, and pharmaceutical industries (Pichersky and Raguso 2018). Especially, terpenoids exert their effects on human health including anticancer, antiviral, anti-inflammation, immune regulation, antioxidation, and other functions (Davies et al. 2015; Hill and Connolly 2020). Due to the good antiviral effects (such as SARS-CoV-2 and hepatitis C virus) (Chao et al. 2016), many terpenoids have been used in the clinical treatment of COVID-19 (Bailly and Vergoten 2020; Murck 2020).

As shown in Fig. 1, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP), the fundamental structural units of all terpenoids, are synthesized through the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. IPP and DAMPP can be reversibly isomerized by isopentenyl pyrophosphate

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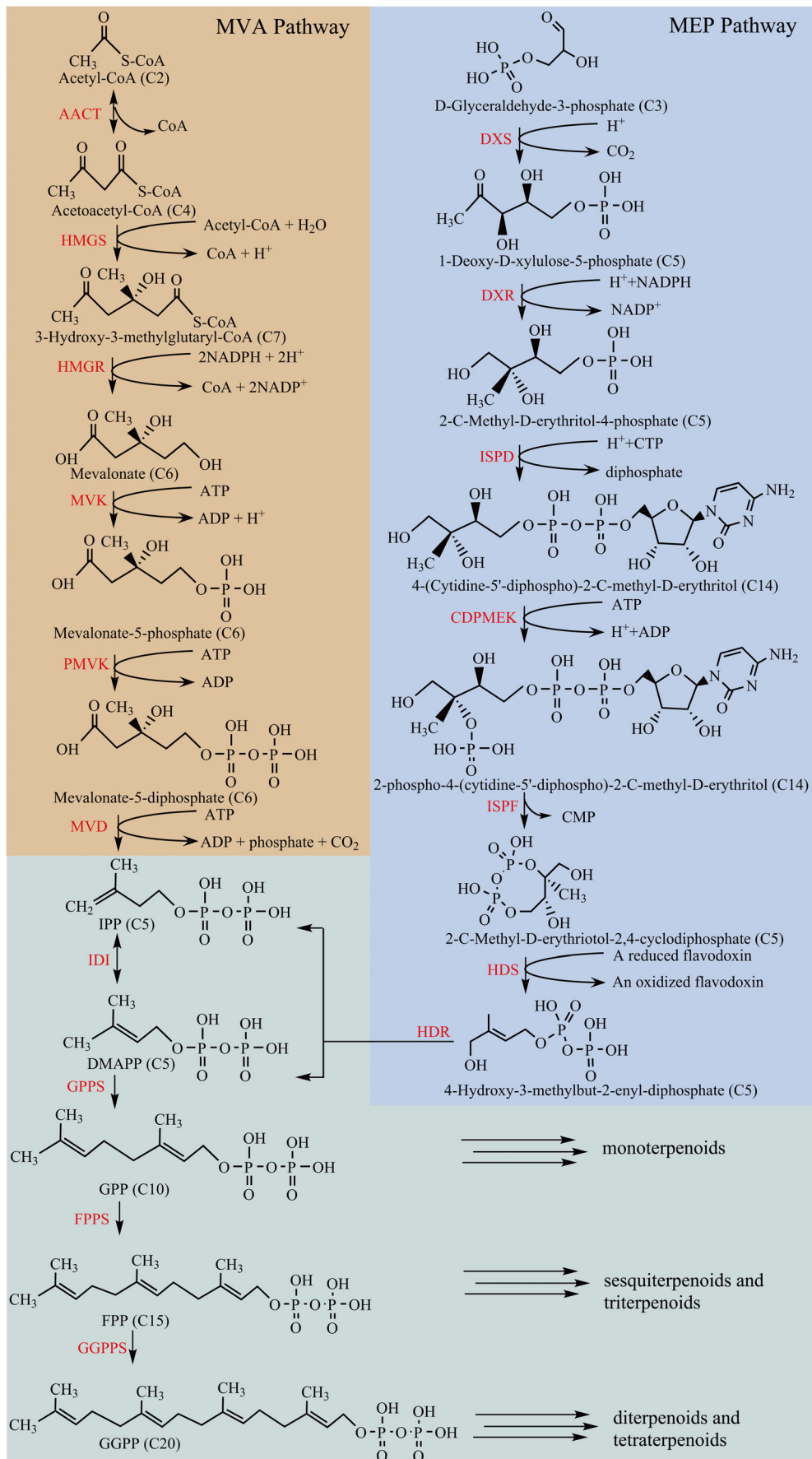
◀ **Fig. 1** Overview of the MVA and MEP pathways for terpenoid production in plant and microorganism. Single arrows represent one-step conversions, and triple arrows represent multiple steps. AACT, acetoacetyl-CoA thiolase; HMGS, hydroxymethylglutaryl-CoA synthase; HMGR, hydroxymethylglutaryl-CoA reductase; MVK, mevalonate kinase; PMVK, phosphomevalonate kinase; MVD, diphosphomevalonate decarboxylase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; ISPD, 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase; CDPMEK, 4-(cytidine 5′-phospho)-2-C-methyl-D-erithritol kinase; ISPF, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; FPPS, farnesyl-diphosphate synthase; HDS: 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase; HDR, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; GPPS, prenyltransferases geranyl diphosphate synthase; FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase

isomerase (IDI). Afterwards, IPP and DMAPP are converted by prenyltransferases to longer chain terpenoid skeletons, such as geranyl diphosphate (GPP, the C_{10} skeleton of monoterpenoids), farnesyl diphosphate (FPP, the C_{15} skeleton of sesquiterpenoids and triterpenoids), and geranylgeranyl diphosphate (GGPP, the C_{20} skeleton of diterpenoids and tetraterpenoids). The various bioactivities of terpenoids can be achieved through structural rearrangement of the carbon skeleton and the addition of functional groups, such as glycosyl, hydroxyl, ketone, carbonyl, and aldehyde (Kirby et al. 2009; Sun et al. 2019).

Previously, terpenoid products were obtained in a general of two ways: plant extraction and chemical synthesis. With the development of biological techniques in recent years, microbial synthesis has become a promising alternative approach in the production of terpenoids (Sun et al. 2020; Yu et al. 2020). Microorganisms grow faster than plants, and the biosynthetic processes are generally sustainable and environmental friendly. However, despite few terpenoids such as artemisinin, an amorphane sesquiterpene which has achieved the goal of industrial production in the genetically modified *Saccharomyces cerevisiae* (Paddon et al. 2013), the biochemical production of most terpenoids is insufficient to be commercial. The main hurdle is the poor inner catalytic activity of plant-derived enzymes in microbes (Sun et al. 2019). To overcome the bottleneck encountered, new breakthroughs need to be found. Some studies suggested that enzymes derived from microbes functioned well in engineered microbes (Huang et al. 2015; Wang et al. 2019; Xu et al. 2018). Considering the long-term harmony and coevolution in plant-endophyte symbioses, endophytes are realized to be potential alternatives for terpenoid bioproduction, either directly or indirectly (Venugopalan and Srivastava 2015). In the present review, research progresses of endophyte in the bioproduction of terpenoids, including endophytic production of terpenoids, the heterologous expression of endophyte-derived terpenoid biosynthetic genes,

the role of endophyte in enhancing their hosts' terpenoid production, and biotransformation of terpenoids by endophytes, were summarized. It is beneficial for researchers to make better understanding and more effective utilization of these excellent resources to achieve efficient synthesis of terpenoids.

Plant endophytes as the alternative sources for terpenoid bioproduction

Plant endophytes are a group of microorganisms that colonize in plant tissues without apparently pathogenic effects to their hosts. Researchers have indicated the presence of one or more types of endophytes in every plant studied to date (Gupta et al. 2020; Shi et al. 2015; Shi et al. 2016). The population of endophytes in a plant is highly variable and depends on various components, including plant genotype, plant growth stage, plant physiological status, the type of plant tissues, the environmental condition of the soil, and different agricultural practices (Gupta et al. 2020). Endophytes exhibit complex interactions with their hosts. For example, they are known to enhance plant growth and nutrient gain, and to improve the tolerances of plants to various types of abiotic and biotic stresses. In addition, it has been proved that endophytes were able to produce some plant-derived high-value compounds, including terpenoids or their precursors (Kharwar et al. 2011; Kusari et al. 2013; Kusari et al. 2014a; Newman 2018; Souza et al. 2011). Over the past decades, many valuable terpenoids with antioxidant, anticancer, and antimicrobial activities have been successfully identified from endophytes (Table 1).

Why could endophytes produce so many phytochemicals like terpenoids and other compounds, while the microbes isolated from other habitats were seldomly able to do so? Some studies considered that the phytochemical biosynthesis functions of endophytes were obtained through their long-term evolution in their hosts by the horizontal gene transfer between plant-associated endophytes and the hosts, as well as among the endophytes (Tiwari and Bae 2020; Zhang et al. 2019), making endophytes as important sources of diverse plant secondary metabolites. In contrast, some other studies showed that the secondary metabolite biosynthetic genes in some endophytes were not homologous with their hosts, and some genes were even absent in the host genomes (Heinig et al. 2013), indicating that the phytochemical-producing endophytes might evolve independently. Therefore, some systematic studies are needed to elucidate why endophytes could produce plant secondary metabolites.

Table 1 Examples of high value terpenoids with relatively high yield isolated from endophytes

Terpenoid	Endophyte	Host plant	Yield	Reference
Azadirachtin A	*Eupenicillium parvum*	*Azadirachta indica*	43 µg/L	(Kusari et al. 2012)
Azadirachtin B	*Eupenicillium parvum*	*Azadirachta indica*	11 µg/L	(Kusari et al. 2012)
Camptothecin	*Fusarium solani* MTCC 9668	*Apodytes dimidiata*	28.9 µg/L	(Venugopalan et al. 2016)
Camptothecin	*Nodulisporium*	*Nothapodytes foetida*	45 µg/g*	(Rehman et al. 2009)
Cryptotashinone	*Penicillium canescens*	*Salvia abrotanoidescan*	0.31 ± 0.12 mg/g*	(Boghsani et al. 2020)
Cryptotashinone	*Penicillium Murcianum*	*Salvia abrotanoidescan*	0.86 ± 0.2 mg/g*	(Boghsani et al. 2020)
Cryptotashinone	*Paraphoma radicina*	*Salvia abrotanoidescan*	1.09 ± 0.29 mg/g*	(Boghsani et al. 2020)
Cryptotashinone	*Coniolariella hispanica*	*Salvia abrotanoidescan*	0.23 ± 0.04 mg/g*	(Boghsani et al. 2020)
Ginsenoside Rg3	*Agrobacterium* sp. PDA-2	*Panax ginseng*	0.062 g/L	(Yan et al. 2019)
Ginsenoside Rh2	*Agrobacterium* sp. PDA-2	*Panax ginseng*	0.019 g/L	(Yan et al. 2019)
Ginsenosides	*Penicillium* sp. G22	*Aralia elata*	2.049 g/L	(Wu et al. 2012)
Huperzine A	*Shiraia* sp. Slf14	*Huperzia serrata*	142.6 µg/g*	(Zhu et al. 2010)
Huperzine A	*Cladosporium cladosporioides* LF70	*Huperzia serrata*	39.61 µg/g*	(Zhang et al. 2011)
Huperzine A	*Colletotrichum gloeosporioides* ES026	*Huperzia serrata*	1 µg/g*	(Shu et al. 2014)
Huperzine A	*Colletotrichum gloeosporioides* ES026	*Huperzia serrata*	45.81 µg/g*	(Zhao et al. 2013)
Huperzine A	*Trichoderma harzianum* L44	*Huperzia serrata*	37.63 µg/g*	(Dong et al. 2014)
Huperzine A	*Paecilomyces tenuis* YS-13	*Huperzia serrata*	21 µg/L	(Su and Yang 2014)
Huperzine A	*Ceriporia lacerata* MY311	*Phlegmariurus phlegmaria*	40.53 µg/L	(Wang et al. 2011)
Huperzine A	*Penicillium* sp. LDL4.4	*Huperzia serrata*	168.9 µg/g*	(Thanh et al. 2019)
Huperzine A	*Fusarium* sp. C17	*Phlegmariurus taxifolius*	3.2 µg/g*	(Cruz et al. 2020)
Huperzine A	*Alternaria brassicae* AGF041	*Huperzia serrata*	42.89 µg/g*	(Zaki et al. 2019)
Huperzine A	*Fusarium* sp. Rsp5.2	*Huperzia serrata*	19.45 µg/g*	(Le et al. 2020)
Saponins	*Fusarium* sp. Pg27	*Panax ginseng*	0.181 g/L	(Wu et al. 2013)
Saponins	*Aspergillus* sp. Pg30	*Panax ginseng*	0.144 g/L	(Wu et al. 2013)
Saponins	*Verticillium* sp. Pg42-1	*Panax ginseng*	0.144 g/L	(Wu et al. 2013)
Tanshinone I	*Trichoderma atroviride*	*Salvia miltiorrhiza* Bunge	1.119 ± 0.008 µg/g*	(Ming et al. 2012)
Tanshinone IIA	*Trichoderma atroviride*	*Salvia miltiorrhiza* Bunge	3.049 ± 0.001 µg/g*	(Ming et al. 2012)
Tanshinone IIA	TR21	*Salvia miltiorrhiza* Bunge	18.827 ± 0.22 g/L	(Zhang et al. 2018)
Taxol	*Phoma medicaginis*	*Taxus wallichiana* var. mairei	PDB culture: 1.215 mg/L, spent culture medium: 0.936 mg/L, dry mycelium: 20 µg/g	(Jian et al. 2017)
Taxol	*Cladosporium* sp. F1	*Taxus baccata*	129 µg/g*	(Kasaei et al. 2017)
Taxol	*Aspergillus aculeatinus* Tax-6	*Taxus chinensis* var. mairei	1337.56 µg/L	(Qiao et al. 2017)
Vinblastine	*Fusarium oxysporum*	*Catharanthus roseus*	76 µg/L	(Kumar et al. 2013)
Vincristine	*Fusarium oxysporum*	*Catharanthus roseus*	67 µg/L	(Kumar et al. 2013)
Vincristine	*Talaromyces radicus*	*Catharanthus roseus*	670 µg/L	(Palem et al. 2015)
Vinblastine	*Talaromyces radicus*	*Catharanthus roseus*	70 µg/L	(Palem et al. 2015)
Vinblastine	*Curvularia verruculosa*	*Cathranthus roseus*	182 µg/L	(Parthasarathy et al. 2020)
Zeaxanthin diglucoside	*Pseudomonas* sp. 102515	*Taxus chinensis*	206 ± 6 mg/L	(Fidan and Zhan 2019)

*µg/g: terpenoid yield per dry weight of mycelium

Heterologous production of endophyte-derived terpenoids

Although various terpenoids can be synthesized by endophytes, all of these endophytes have not been suitable for commercial application due to the low yield and the weakening biosynthetic capacity upon repeated subcultivation. Development of techniques in metabolic engineering and synthetic biology promoted the idea of expressing the terpenoid biosynthetic pathway in industrial microbes (Belcher et al. 2020). Besides, system biology techniques enlarged the bank of potential genes involved in terpenoid biosynthesis from environments. For example, modern metagenomic

sequencing approaches and de novo assembly of microbial genomes from metagenome data provide powerful strategies in discovering the novel microbes and genes involved in terpenoid biosynthesis from the endosphere and rhizosphere microbiomes, regardless of whether the microbes are culture-dependent or culture-independent (Carrion et al. 2019).

Here, by taking Huperzine A (HupA) as an example, we discuss the feasibility of heterologous production of endophyte-derived terpenoids. HupA is a sesquiterpene alkaloid naturally existing in members of the Huperziaceae, such as *Huperiza serrata*. A large number of clinical trials have shown that HupA is an effective therapeutic medicine with

minor side effects for Alzheimer's disease (AD) due to its high anti-acetylcholinesterase activity (Zhao et al. 2013). So far, part biosynthetic pathway of HupA in the family Huperziaceae has been illustrated (Fig. 2a). The first two steps are catalyzed by lysine decarboxylase (LDC) and copper amine oxidase (CAO), which convert L-lysine to 5-aminopentanal, the precursor of HupA. The genes expressing LDCs and CAOs have been identified from *Lycopodium clavatum* (Bunsupa et al. 2016) and *H. serrata* (Xu et al. 2017), but their enzymatic promiscuity activities were strong. Many other enzymes participating in the HupA biosynthetic pathway remain to be identified.

The discovery of HupA biosynthetic genes from endophytes related to family Huperziaceae is of great value for both scientific research and commercial applications. In 2010, two HupA-producing endophytic fungi *Shiraia* sp. Slf14 (Zhu et al. 2010) and *Cladosporium cladosporioides* LF70 (Zhang et al. 2011) were isolated from the leaves of *H. serrata*, and their yields of HupA were 142.6 μg/g and 39.61 μg/g dry mycelium, respectively. Afterwards, Zhu and colleagues sequenced the whole genome of *Shiraia* sp. Slf14, identified a putative HupA biosynthetic gene cluster (Yang et al. 2014), and then heterogeneously expressed the *SsCAO* gene of the gene cluster into *Escherichia coli* (Yang et al. 2016). They found that the genetically modified *E. coli* strain was able to convert cadaverine to 5-aminopentanal. In 2014, another HupA-producing fungal endophyte *Colletotrichum gloeosporioides* ES026 was isolated from *H. serrata* with the HupA yield of 45.81 μg/g dry mycelium at most (Zhao et al. 2013). Shu and colleagues did de novo RNA sequencing of *C. gloeosporioides* ES026 and genes encoding LDC (*CgLDC*) and CAO (*CgCAO*) were identified (Zhang et al. 2015). Later on, they heterogeneously overexpressed *CgLDC* and *CgCAO* in *E. coli*, and successfully obtained 5-aminopentanal in cells (Zhang et al. 2017) (Fig. 2b).

Besides HupA, the microbial-based production of some other terpenoids was supported by genetic information of endophytes. For example, John M. Gladden and colleagues discovered 26 putative terpene synthases (TPSs) derived from four endophytic fungal strains (*Daldinia eschscholzii* EC12, *Hypoxylon* sp. CO27, *Hypoxylon* sp. CI4A, and *Hypoxylon* sp. EC38), of which 12 were functionally expressed in *E. coli* and induced the production of a wide variety of monoterpenoids and sesquiterpenoids (Wu et al. 2016). Liu and colleagues first identified and described a chimeric diterpene synthase from the endophyte *C. gloeosporioides* ES026 as (5R,12R,14S)-dolasta-1(15),8-diene synthase (CgDS), the chimeric fungal clade II-D terpene synthases and catalyzes a C1-III-IV cyclization, and obtained this compound with the titer of 7.3 mg/L in *S. cerevisiae* (Bian et al. 2018). Zhan and colleagues isolated an endophytic bacterium *Pseudomonas* sp. 102515 that could produce zeaxanthin diglucosidea, a promising antioxidant terpenoid that belongs to the family of

carotenoids, from the leaves of *Taxus chinensis*, and then amplified a carotenoid biosynthetic gene cluster of this strain in *Pseudomonas putida* KT2440, resulting in the yield of zeaxanthin diglucoside at 121 mg/L (Fidan and Zhan 2019).

Although it is technically available to express endophyte-derived terpenoid biosynthetic genes in engineered strains, it seems that the yield of terpenoids is still short of the commercial expectations. It is expected that further improvement of terpenoid production can be achieved through metabolic engineering combined with protein engineering.

Endophytes with the ability to enhance their hosts' terpenoid production

Endophytes could not only produce terpenoids by themselves, but also stimulate the terpenoid accumulation in their host plants. For example, tanshinones, a golden group of diterpene quinones with the pharmacological effects, like antitumor, antioxidation, anti-inflammation, cardiovascular and cerebrovascular protection, are the major lipophilic ingredients of *Salvia miltiorrhiza* Bunge (Danshen) (Dong et al. 2011). To date, people have identified more than 40 tanshinones from the chemical constitutes of *S. miltiorrhiza*, such as tanshinone I-VI, cryptotashinone, isotanshinone I-II, and danshenol A, but their biosynthetic pathways in *S. miltiorrhiza* are only partially elucidated (Guo et al. 2016) (Fig. 3). In 2012, a tanshinone-producing endophytic fungus *Trichoderma atroviride* D16 was isolated from *S. miltiorrhiza* root, which can produce tanshinones I and IIA in rich mycological medium (Ming et al. 2012). One year later, Qin and colleagues found that *T. atroviride* D16 could promote the cell growth and tanshinone production in *S. miltiorrhiza* hair roots, through the transcriptional regulation by the polysaccharide fraction (PSF) secreted from *T. atroviride* mycelium (Ming et al. 2013). According to further analysis via infrared (IR) and nuclear magnetic resonance (NMR), the key components in PSF responding for boosting tanshinone production were mannose, glucose, and galactose (Wu et al. 2019). According to the proteomics analysis of the *S. miltiorrhiza* hairy roots exposed to PSF, the tanshinone biosynthesis induced by PSF in *S. miltiorrhiza* hairy roots may be correlated with peroxide reaction, Ca^{2+} triggering, jasmonic acid (JA) signal transduction, and protein phosphorylation, finally resulting in an increasement of leucine-rich repeat (LRR) protein synthesis (Peng et al. 2019). Another endophytic fungus *Chaetomium globosum* D38 isolated from the roots of *S. miltiorrhiza* induced its host to produce more tanshinones, especially for dihydrotanshinone I and cryptotanshinone, through upregulating the expression of key genes involved in tanshinone biosynthetic pathway (Zhai et al. 2018).

There are some other examples to show how endophytes promote the terpenoid production in their hosts. Alok Kalra
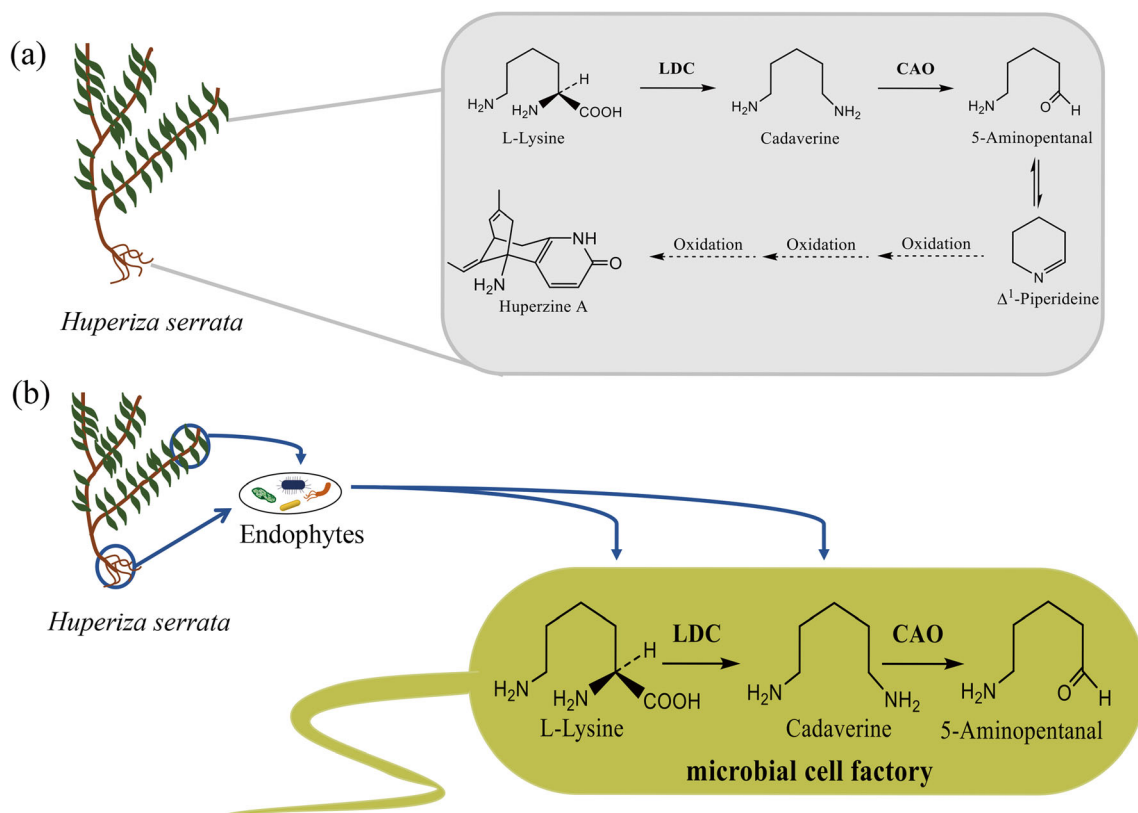
Fig. 2 The biosynthetic pathway of HupA. **a** The proposed biosynthetic pathway of HupA in members of the Huperziaceae. **b** The heterogeneous expression of HupA synthetic genes from endophytes in microbial cell factory. LDC, lysine decarboxylase; CAO, copper amine oxidase. Solid arrows indicate the established relationships, and dashed arrows indicate hypothetical relationships

and colleagues isolated a few nitrogen-fixing root-associated and indole-3-acetic acid (IAA)–producing endophytes from different parts of the medicinal plant *Withania somnifera*, and found that they could induce their hosts to produce more withaferin-A in roots, one of the major phytochemical triterpenoid derivatives in *W. somnifera*, by inducing IAA production and increasing the transcriptional activity of withanolide biosynthesis genes in roots, especially MEP-pathway genes (*DXS* and *DXR*) (Pandey et al. 2018). Zhang and colleagues screened out an endophytic bacteria *Bacillus pumilus* from the medicinal herb *Glycyrrhiza uralensis* Fisch. They found that *B. pumilus* can improve *G. uralensis* growth under drought stress through the modification of antioxidant accumulation and enhance glycyrrhizic acid content by the incremental expression of key enzymes, such as squalene synthase, 3-hydroxy-3-methylglutaryl CoA reductase, and beta-amyrin synthase (Xie et al. 2019).

In summary, cells or fragments of endophytes could play the role of elicitors to induce the formation of bioactive compounds, such as terpenoids, in plant or cell suspension cultures. This endophyte-induced bioactive compound synthesis in plant cells is considered through the signal transduction process, which is composed of several steps including recognition of elicitors, signal transduction, integration with transcription factors, and gene activation (Zhai et al. 2017). In

detail, endophytic elicitors, such as fungal proteins, oligosaccharides, and polyunsaturated fatty acids (Wu et al. 2019), could activate the signal transduction pathways in plants, including ion fluxes and Ca^{2+} signaling pathway, nitric oxide (NO) signaling pathway, reactive oxygen species (ROS) signaling pathway, salicylic acid (SA) signaling pathway, JA signaling pathway, and their cross-talking. Then, the produced signal molecules could integrate with transcription factors which regulate the expression of bioactive compound–associated functional genes (Zhao et al. 2005), leading to the significant accumulation of bioactive compounds in plant cells (Fig. 4).

Although the outline of endophyte-induced plant terpenoid synthesis has made important progress, the detailed mechanisms are not declared yet. For example, how the various signaling molecules regulate the expression of transcription factors during the endophyte-induced accumulation of terpenoids in medical plants is scarcely investigated. Han and colleagues proved that class I TGA transcription factors combined with methyl jasmonate could increase the production of triptolide and two sesquiterpene pyridine alkaloids, but the detailed mechanism has not been revealed (Han et al. 2020). Moreover, novel endophyte-derived elicitors, signal molecules, transduction pathways, transcriptional factors linking with in situ terpenoid metabolism, and the docking

Fig. 3 Partial pathways for tanshinone biosynthesis and chemical structures for some representative tanshinones. CPS, copalyl diphosphate synthase; KSL, kaurene synthase-like; CYP, cytochrome P450 monooxygenase
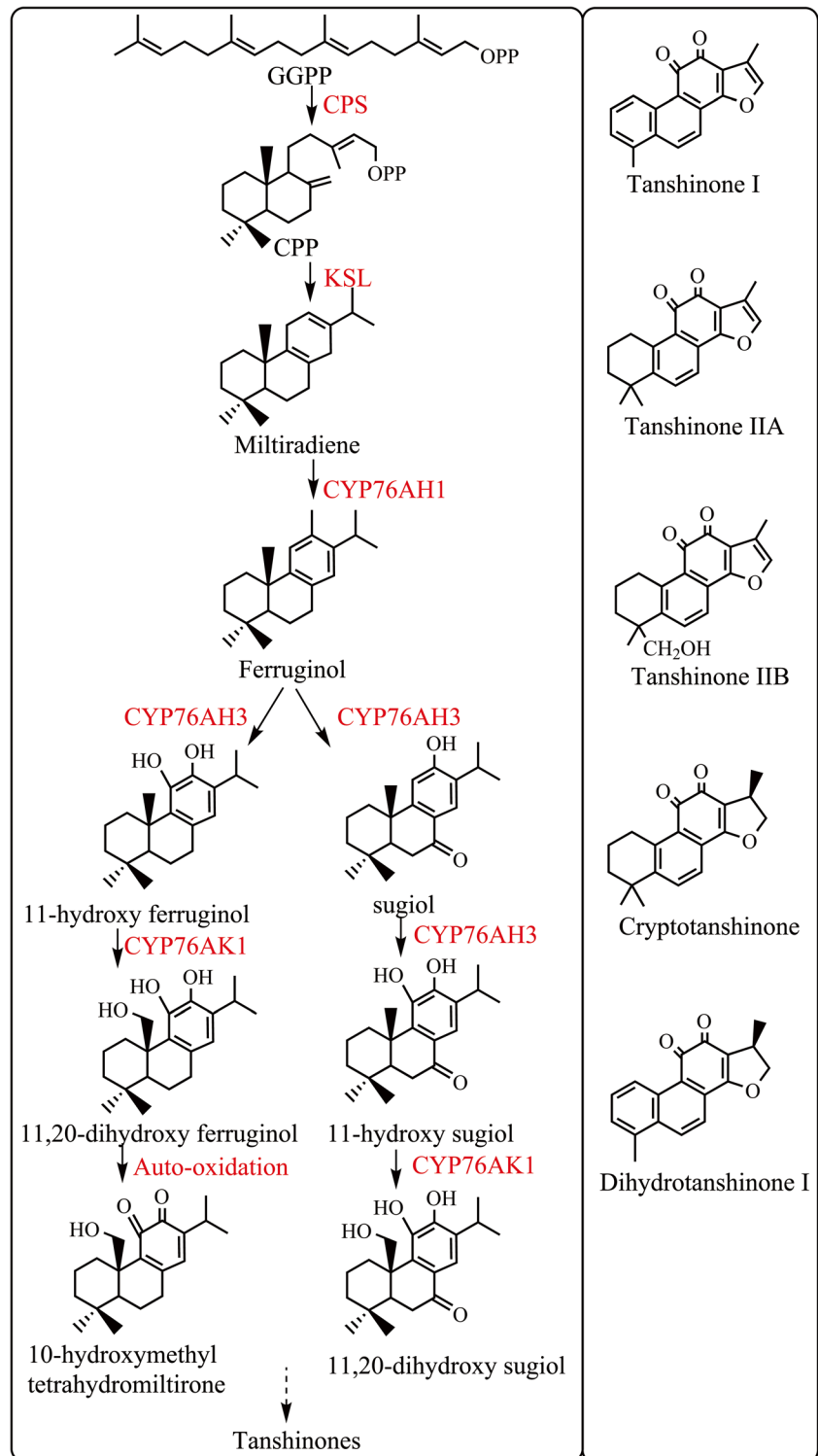
processes between elicitors and the corresponding receptors are not elaborated. Benefiting from the technological development in synthetic biology and botany, plant synthetic biology is regarded as another hot spot (Nemhauser and Torii 2016). Nonetheless, the development of plant synthetic biology is still at an initial stage due to the insufficiency of quantificationally standardized components and incompatibilities between standardized components and plant system (Patron et al. 2015). In the future, researchers should focus on the studies of the key transcription factors related to the endophytic elicitors. Only when key transcription factors screened out can they be transferred to the corresponding

medical plants or other model plants to increase the accumulation of the secondary metabolites greatly. In addition, despite that a variety of synthetic sensors has been used in the study of plant endogenous signaling pathways, complex genetic circuits have not been realized in plants. So future plant synthetic biology development depends on a large degree of basic research breakthrough.

Biotransformation of terpenoids by endophytes

Besides the function of terpenoid biosynthesis directly or indirectly, endophytes have the potential with the function of terpenoid decoration to change their structures and bioactivities. Currently, some endophytes have been utilized to produce useful enzymes, which have significant regio- and stereo-selectivities, for the production of terpenoid derivatives (Corrêa et al. 2014).

Ginseng is an important pharmaceutical herb belonging to Araliaceae family. It has been utilized in Chinese medicine for thousands of years. Ginsenosides are the most valuable and major active triterpenoids in ginseng species with the therapeutic effects of anti-tumor, anti-age, and hepatitis therapeutic effectiveness. The biosynthetic pathway of ginsenosides has been elucidated (Wang et al. 2020) (Fig. 5a). They are composed of major ginsenosides (Rb1, Rb2, Rc, Rd, Rg1, etc.) and rare ginsenosides (Rg3, Rh1, Rh2, F2, compound K, etc.). Compared with major ginsenosides, rare ginsenosides (deglycosylated

ginsenosides) are more pharmaceutically active, because they have relatively smaller sizes and are easily able to penetrate cell membranes (Xu et al. 2003). Considering that rare ginsenosides are too few to be purified from most natural ginseng plants, it is of great significance to study the conversion of major ginsenosides into rare ginsenosides (He et al. 2019). It was found that ginsenoside Rb1, the main active ingredient of *Panax Notoginseng*, could be deglycosylated to form ginsenoside F2 and compound K by the glucosidase of the endophytes *Fusarium* sp. YMF1.02670 and YMF1.02193 (Luo et al. 2013) (Fig. 5b). Yin and colleagues screened out 32 β-glucosidase-producing endophytes from *Platycodon grandiflorum*, among which *Luteibacter* sp. JG09 can effectively convert ginsenosides Rb1, Rb2, Rc, and Rd into rare ginsenosides F2 and compound K, and convert ginsenoside Rg1 into rare ginsenoside Rh1 (Cui et al. 2016) (Fig. 5b). The maximum production rate of ginsenosides F2 and compound K reached 94.53% and 66.34%, respectively. Later on, they successfully isolated another β-glucosidase-producing endophytic bacterium *Burkholderia* sp. GE 17-7 from *P. ginseng* roots with the capability of converting ginsenoside Rg3 from Rd1 (Fu et al. 2017) (Fig. 5b). Accordingly, further studies to identify and modify various β-glucosidases from endophytes have the potential to effectively increase ginsenoside bioproduction.

Endophytes have potential biotransformation activities on many other terpenoids. For example, ursolic acid is a pentacyclic triterpenoid with the anti-inflammatory, anti-
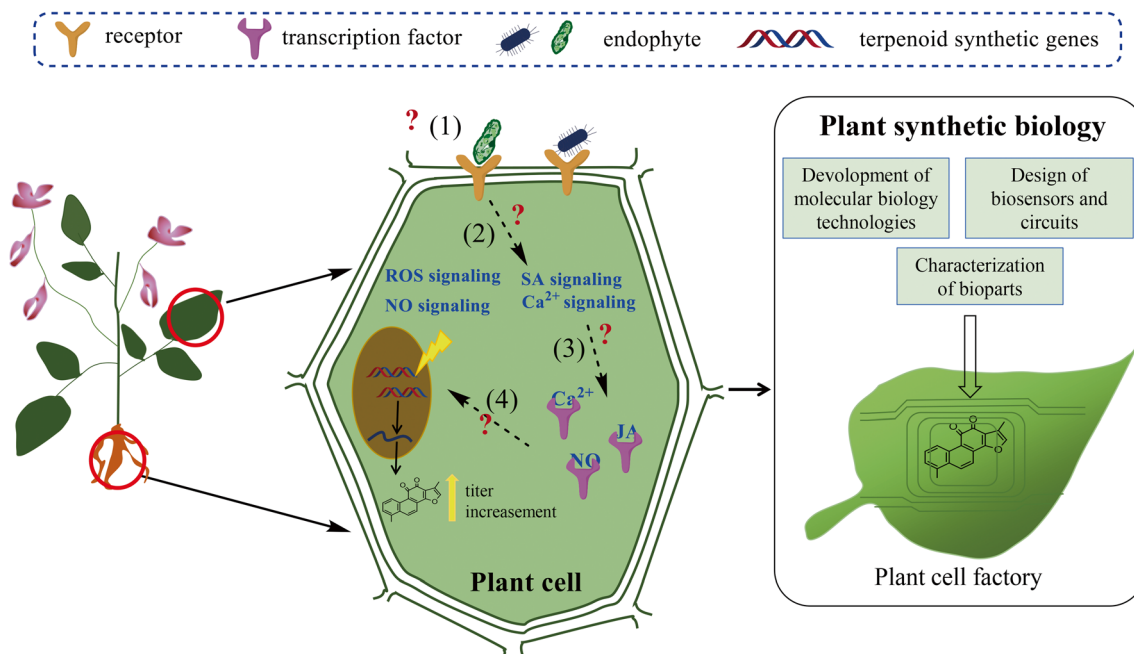

Fig. 4 The process of endophyte-induced secondary metabolite synthesis in plant cells. (1) Recognition of elicitor; (2) signal transduction; (3) integration with transcription factors; (4) gene activation; ROS, reactive oxygen species; JA, jasmonic acid; SA, salicylic acid; NO, nitric oxide
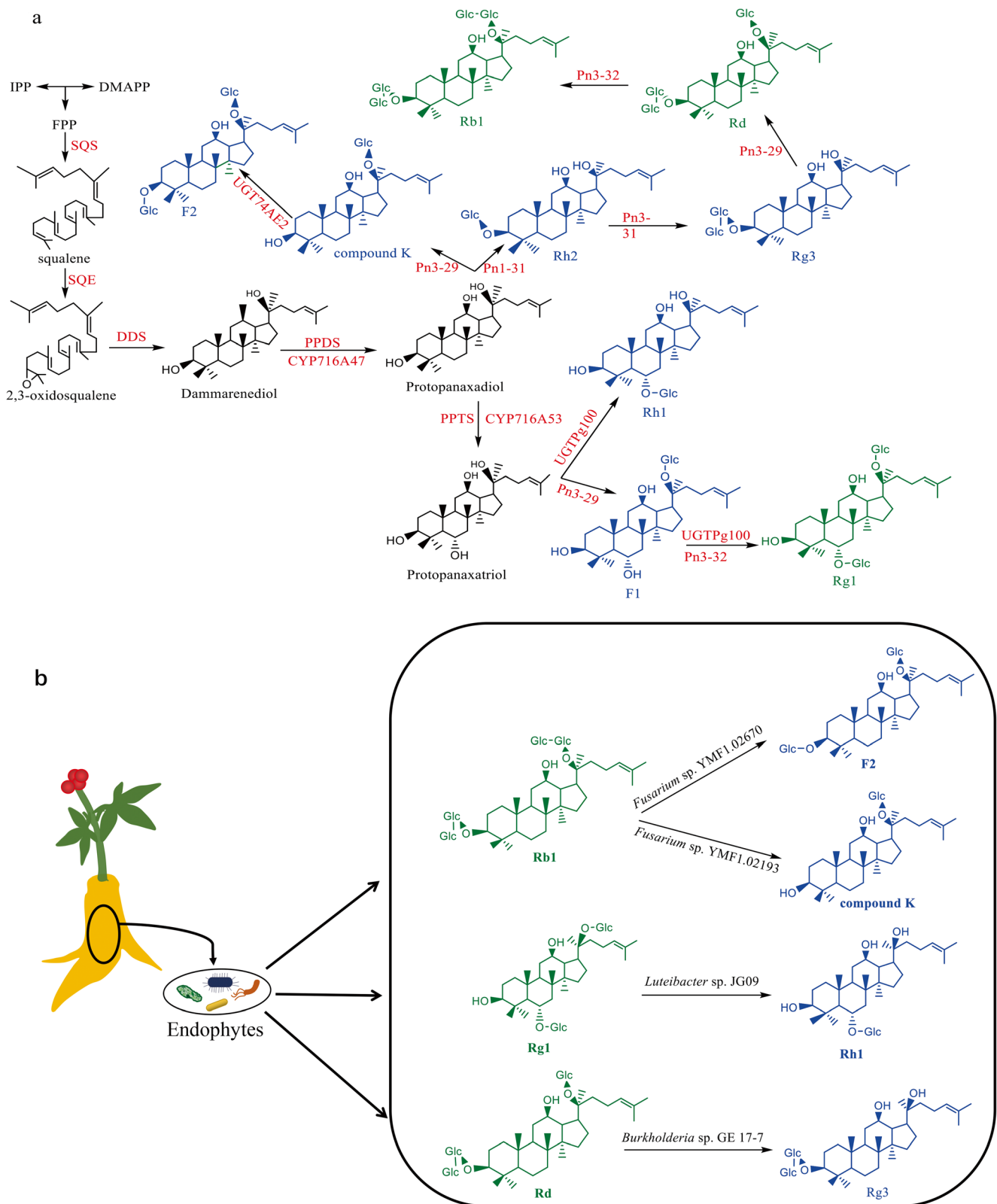
Fig. 5 The proposed biosynthetic pathway of ginsenosides. **a** Key enzymes and intermediates involved in ginsenoside biosynthesis. **b** Examples of biotransformation of ginsenosides by endophytes. SS, squalene synthase; SE, squalene epoxidas; DDS, dammarenediol-II synthase; PPDS, protopanaxadiol synthase; PPTS, protopanaxatriol synthase; CYP450, cytochrome P450 monooxygenase; UGT, uridine diphosphate glycosyltransferase. Green compounds represent major ginsenosides; blue compounds represent rare ginsenosides

microbial, and antiviral activities. Some of its derivatives are very important for the structure-activity relationship study, but they are hard to be obtained through the chemical modification of ursolic acid since ursolic acid has limited active sites. Thanks to the investigation on endophytes, the fungal endophytes isolated from *H. serrata*, *Pestalotiopsis microspora*, and *Umbelopsis isabellina* were reported to be able to transform ursolic acid to new compounds by structural modification through maybe the cooperation of transferases and esterases (Fu et al. 2011a, b). A second example is on the gentiopicroside (GPS), a monoterpenoid glucoside whose pharmacological properties could be activated after the enzymatic or acidic hydrolysis (Zeng et al. 2014). An endophytic fungus *Penicillium crustosum* 2T01Y01, isolated from a medicinal plant *Dendrobium candidum* Wall. ex Lindl., had a high GPS-transforming ability, and could produce three known and four novel deglycosylated GPSs (Zeng et al. 2014). It was proposed that the GPS metabolic pathways in *P. crustosum* 2T01Y01 mainly include deglycosylation, hydrolyzation, cyclization, reduction and hydrogenation, or oxidation and decarboxylation. The authors speculated that the deglycosylation by the β-glucosidase existing in the fungus might be the initiation step, but the enzymes involved in the other steps need to be elucidated.

In summary, endophytes can be efficiently used for the biotransformation of natural compounds through their special enzymes. The biotransformation reactions mainly include hydroxylation, hydrolysis, reduction, oxidation, epoxidation, O-methylation, ring-expansion, isomerization, and methyl migration reactions. Considering that rational design and directed evolution of enzymes could accelerate the improvement of their specificities, stabilities, and/or efficiencies, these strategies could be used to ultimately improve the conversion efficiencies of biotransformational enzymes in the future.

Conclusions and future perspectives

Endophytes are a treasure trove of terpenoid biosynthesis through direct or indirect manners. Nevertheless, investigations on endophyte-related terpenoid synthesis are still in their infancy. To achieve their commercial applications in industries, several serious challenges should be overcome. Firstly, the lifestyles and genetic systems of most endophytes are poorly understood, which have hampered our in vivo genetic manipulations. Secondly, the heterologous expression of endophyte-derived genes within the normal microbial cell factories is usually in face with instability, low enzymatic activities, misfolding, incorrect post-translational modification, a mass of crosstalk between the endogenously primary metabolism and the artificial metabolic pathways, and so on (Kusari et al. 2014b). Thirdly, if the target endophytes prefer to make steady

functions in plant-associated microbiomes through physical and/or metabolic interactions, their in vitro monocultures might be in face with the gradual reduction in terpenoid yields. With the development of the multi-omics approaches and bioinformatics, exploring, identifying, and characterizing the genetic and metabolic elements involved in the terpenoid synthesis in endophytes have been advancing. With further development and the combination of enzyme engineering, pathway optimization, molecular techniques, and some other modern technologies, endophytic-derived terpenoid bioproduction could be boosted furthermore.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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