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•Review•

Microbes as a production host to produce natural active compounds from mushrooms: biosynthetic pathway elucidation and metabolic engineering

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[ABSTRACT] Mushrooms are abundant in bioactive natural compounds. Due to strict growth conditions and long fermentation-time, microbe as a production host is an alternative and sustainable approach for the production of natural compounds. This review focuses on the biosynthetic pathways of mushroom originated natural compounds and microbes as the production host for the production of the above natural compounds.

[KEY WORDS] Mushroom; Natural compound; Microbes; Synthetic biology; Metabolic engineering
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Introduction

Mushrooms, an attractive delicacy characterized by their unique flavor, taste, and potential health benefits, have been long used for medicinal purposes in Asian countries. "Mushroom" is not a taxonomic category. Actually, from a taxonomic point of view, most species of Basidiomycetes and some species of Ascomycetes belong to mushrooms [1]. Mushrooms are known to be prolific producers of structurally diverse, natural bioactive compounds, and some of them possess anticancer, antiviral, antimicrobial, and antifungal properties [1-3]. These bioactive compounds can be classified into several chemical groups, such as polysaccharides, terpenoids, and particularly sesquiterpenes [4]. However, many mushroom strains are difficult to grow under laboratory conditions, due to their restrict requirements for suitable growth conditions and long fermentation time. Furthermore, extraction of these compounds from natural sources is often costly with respect to the presence of various isoforms and structurally related impurities in complex mixtures [5] and can not meet the demands of today's market [6]. Therefore, it is necessary to explore alternative and sustainable sources of natural compounds. One of the practical methods is to use microbes as a production host to produce natural compounds.

Escherichia coli, *Saccharomyces cerevisiae* and *Xanthophyllomyces dendrorhous* are platform organisms that act as industrial cell factories for the production of a wide variety of compounds ranging from pharmaceutically active substances to food ingredients and biofuels [7]. Production hosts friendly adopt the heterogenous biosynthetic pathways of natural compounds from most genera to yield various natural compounds including polyketides, terpenes, peptides, and alkaloids [8-11]. This approach usually gives a sustainable supply of natural compounds from cultures (0.1–1.0 L). For large scale-production of, for instance, artemisinin and its precursor artemisinic acid, proper engineering methods, such as enzyme engineering, metabolic flux optimization and system optimization have been introduced to improve the titer for industrial use. Currently, heterologous expression is recognized as one of the powerful methods for natural compound production.

Up to 2020, increasing studies have been performed concerning the heterologous production of mushroom metabolites. This review focuses on the production of mushroom originated natural compounds, including copaene, cubebol, ergothioneine, illudins, lagopodins, psilocybin and viridiflorol

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in organisms, and their biosynthetic pathways. Some interesting information is also provided. These findings demonstrate the great potential of mushroom originated natural active compounds in microbes, which will promote the research and development of mushroom originated natural active compounds and diverse pathways for the synthesis of natural compounds.

Copaene

Copaene possesses important biological properties, such as anticarcinogenic and antioxidant activities in the treatment of neurodegenerative diseases [12], or serves as an insect attractant [13]. Through Basic Local Alignment Search Tool (BLAST) analysis of Basidiomycota *Coniophora puteana* genome, which is classified as a common wood rotting fungus, with conserved terpene synthases sequences, Copu2 is proved to show copaene synthase activity [14]. To produce copaene in *E. coli*, Mischko *et al*. heterologously expressed Copu2 to enhance the expression of copaene synthase and two bottleneck enzymes DXS and idi from the native non-mevalonate pathway (MEP) to increase the precursor supply, obtaining an optimized productin of *β*-copaene at 215 mg·L^{-1} [15] (Fig. 1).

Cubebol

Cubebol is of interest to the cosmetic and flavor industry as a registered product with pronounced cooling effects [16], which can be formulated into dietary supplements and flavoring agents [16]. So far, different sources of cubebol synthase have been identified (Table 1). Cop4, originated from *C. cinereus*, was designated as cubebol and *β*-copaene synthase. Recombinant Cop4 was reported to generate 30% *β*-copaene and 10% cubebol with respect to the total terpene production titer [17]. Even under optimized *in vitro* conditions, Cop4 did not generate more than 34.2% cubebol [18]. *Vitis vinifera* originated *δ*-cadinene synthase VvPNCuCad, encoding a multi-product STPS, showsed 20.5% cubebol selectivity when expressed in *E. coli* [19]. Other fungal STPSs with related cubebol synthase activity were cloned and characterized from *A. cinnamomea* (AcTPS9) [20] and *Stereum hirsutum* (Stehi_128017) [21]. From *Coniophora puteana*, Copu3 was identified to show cubebol synthase activity [15] (Fig. 2A).

The first biotechnological production approach reported for the quantitative generation of cubebol utilized a patented plant enzyme (CQ813505.1 from grapefruit *Citrus x paradisi*; 28% cubebol selectivity) but only provided titers of 10 mg·L^{-1} [22]. Mischko *et al*. heterologously expressed Copu3 to enhance the expression of cubebol synthase and two bottleneck enzymes DXS and idi from native non-mevalonate pathway (MEP) to increase the precursor supply, achieving an optimized *δ*-cubebol at 497 mg·L^{-1} [15] (Fig. 2B).

Ergothioneine

Ergothioneine (ERG), a histidine-derived thiol compound, was shown in *in vitro* experiments to function as an antioxidant [23] and cytoprotectant, resulting in its use in dietary supplements and as a cosmetic additive [24]. Mushrooms have been long used as the source of ERG [25]. ERG biosynthetic genes are present in some microbes, such as actinobac-

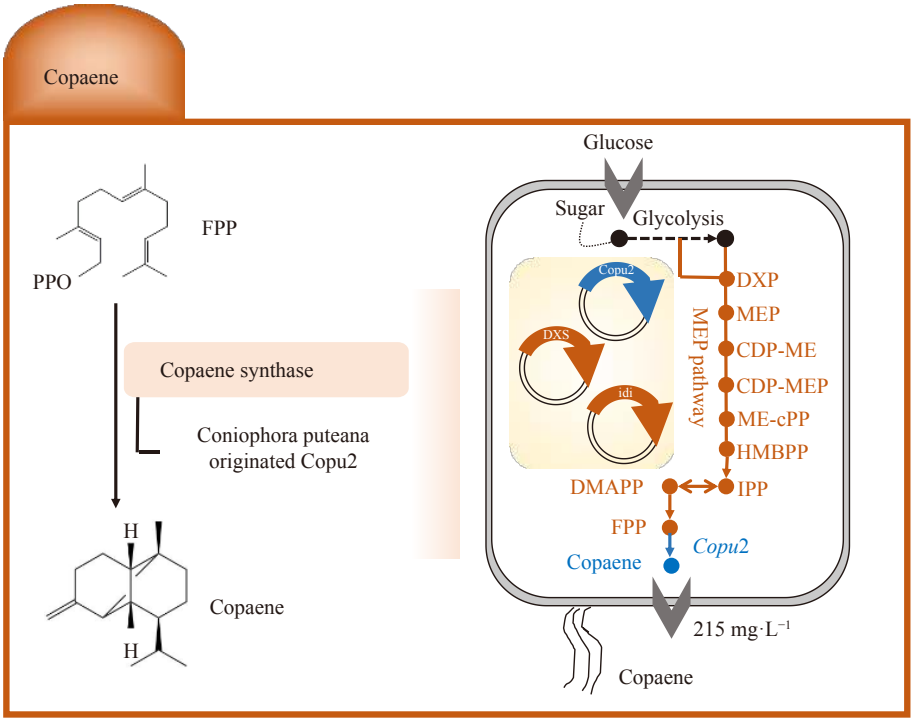

Fig. 1 Diagram of copaene biosynthesis pathway and *E. coli* as the production host for copaene production

Table 1 List of genes in the biosynthesis pathways of the above natural products

Natural products	Genes	Names	Accession No.	Sources
Copaene		Copu2	XP_007771895.1	*C. puteana*
		Cop4	A8NU13.1	*C. cinereus*
Cubebol	Cubebol synthase		XP_007299839	*S. hirsutum*
		BvCS		*B. vibrans*
		Copu3	XP_007765978.1	*C. puteana*
		VvPNCuCad	HM807407.1	*V. vinifera*
		Cop4	A8NU13.1	*C. cinereus*
		Stehi_128017		*S. hirsutum*
		AcTPS9		*A. cinnamomea*
Ergothioneine		*egtA*	433650597	*M. smegmatis*
		egtB	433650596	*M. smegmatis*
	Amidohydrolase	*egtC*	433650595	*M. smegmatis*
		egtD	433650594	*M. smegmatis*
	PLP-dependent C-S lyase	*egtE*	433650593	*M. smegmatis*
Illudins	Protoilludane synthase	*Pro1*	KC852198	*A. gallica*
		OMP6	MUStwsD_GLEAN_10003820	*O. olearius*
		OMP7	MUStwsD_GLEAN_10000831	*O. olearius*
		Stehi1|25180	NW_006763134.1	*S. hirsutum*
		Stehi1|64702	NW_006763145.1	*S. hirsutum*
		Stehi1|73029	NW_006763132.1	*S. hirsutum*
		PpSTS-08	LC378430	*P. placenta*
		PpSTS-18	LC378436	*P. placenta*
		Agr6	MN146029	*A. aegerita*
		Agr7	MN146030	*A. aegerita*
		Denbi1_659367		*D. bispora*
		Hetan2_454193		*H. annosum*
		Hypsu1_138665		*H. sublateritium*
	P450 monooxygenase	P450 6i	MUStwsD_GLEAN_10003819	*O. olearius*
Lagopodins	Cuprenene synthase	Cop6		*C. cinerea*
	P450 monooxygenase	Cox1		*C. cinerea*
		Cox2		*C. cinerea*
Psilocybin	Tryptophan decarboxylases	PsiD		*P. cubensis* and *P. cyanescens*
	Kinase	PsiK		
	Methyltransferase	PsiM		
	P450 monooxygenase	PsiH		
Viridiflorol	Viridiflorol synthase	Agr2	MN146025	*A. aegerita*
		Agr5	MN146028	*A. aegerita*
		Sphst_47084		*S. stellatus*
		Denbi1_816208		*D. bispora*

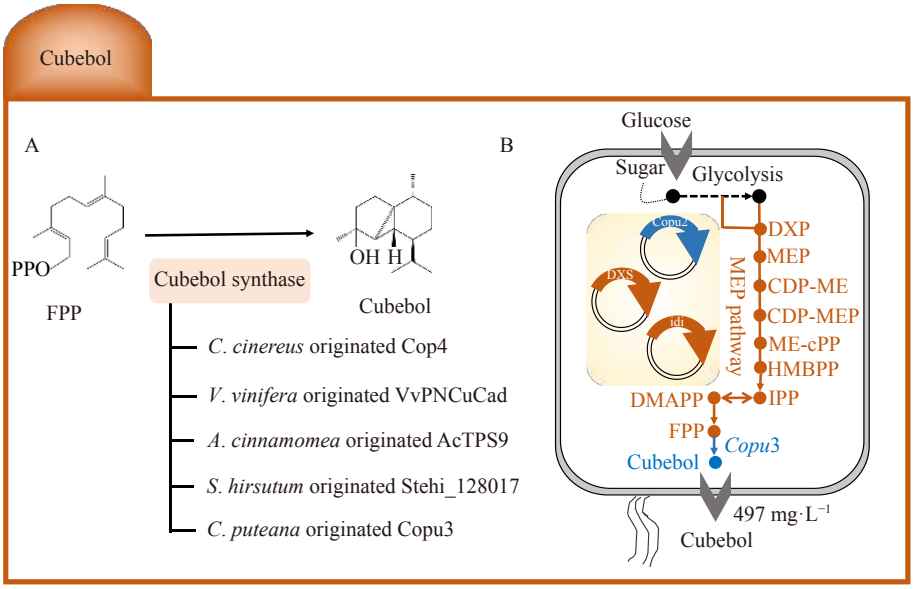

Fig. 2 Diagram of cubebol biosynthesis pathway and *E. coli* as the production host for cubebol production

teria, cyanobacteria, and *α*-proteobacteria, according to the review by Jones *et al*. [26]. Seebeck *et al*. identified *egtABCDE* as the ERG biosynthetic gene cluster from mycobacteria and sucessfully reconstituted this biosynthetic process *in vitro* using recombinant proteins from *Mycobacterium smegmatis* [27] (Fig. 3A). Osawa *et al*. first expressed the egt genes as the operon with tac and T7 promoters *in vivo* and successfully produced ERG, but the production yeild was low. They explained that EgtB-catalyzed reaction was a bottleneck through protein expression assay and enzyme activity assay of each egt genes. Reinforcement of the *γ*GC supply, substrate of EgtB resulted in an increase of ERG production to 24 mg·L^{-1} [28] (Fig. 3B).

Illudin

Illudins are a group of sesquiterpene antibiotics widely reported as antibacterial and antitumor agents [29]. Several species of mushroom-forming fungi (Basidiomycetes) have been reported to produce different types of illudins (Fig. 4A) [30-37]. Illudins M and S are the "stars" among illudins. Illudin S was tested by the National Cancer Institute as a potential antitumor agent against a variety of rodent solid tumors and leukemias. Illudin M is a cytotoxic fungal illudane sesquiterpene comprising strongly electrophilic spirocyclopropane and enone systems. Although illudins are highly effective against cancer cells, their indiscriminate toxicity limits its clinical applications [38]. Fortunately, they are readily available in gram quantities and lend themselves to chemical modification, which can broaden their therapeutic index and enhance cancer selectivity. Analogues of illudins with reduced toxicity have been developed which may be safe, potent antiviral compounds [39]. Oxidation of illudin M with pyridinium dichromate (PDC) afforded dehydroilludin M which showed an improved therapeutic index in animal stud-

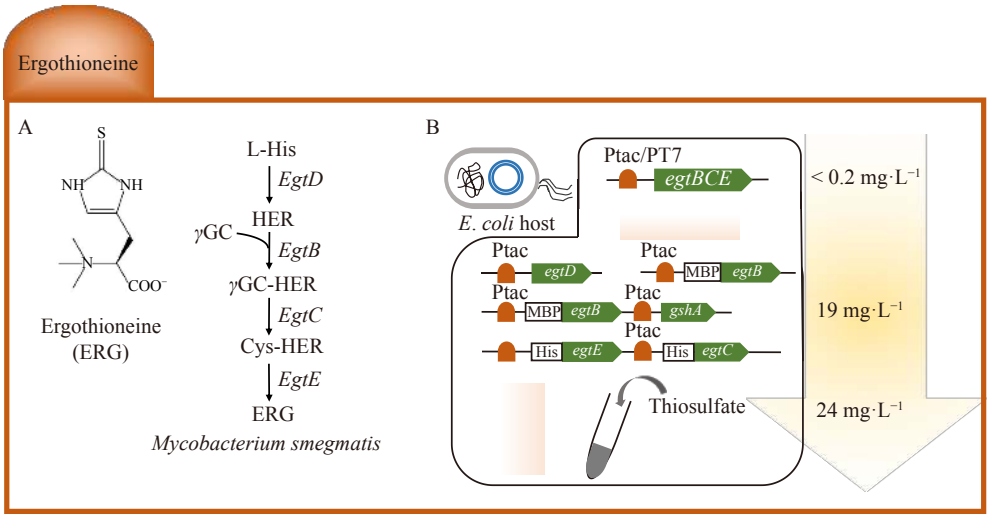

Fig. 3 Diagram of ERG biosynthesis pathway and *E. coli* as the production host for ERG production

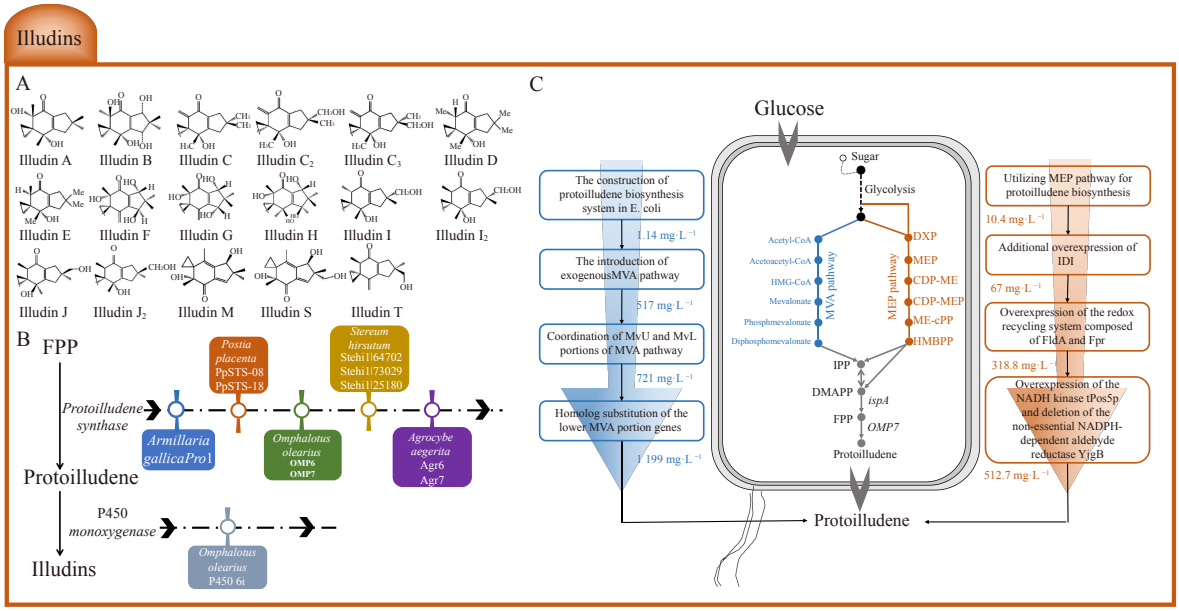

Fig. 4 ***E. coli*** **as the production host for illudin production. A. The structural formula of different types of illudins. B. The diagram of illudins biosynthesis pathway. C. The metabolic engineering of** ***E. coli*** **for the production of illudin precursor protoilludene**

ies [40]. In contrast, Illudin S was tested by the National Cancer Institute as a potential antitumor agent against a variety of rodent solid tumors and leukemias. HMAF, prepared from illudin S *via* a reverse Prins reaction using formaldehyde and H_2SO_4, displayed *in vitro* and *in vivo* antitumor activity and is currently evaluated in a phase I clinical trial [41-42]. Moreover, irofulven is known to inhibit the synthesis of DNA by blocking the cell cycle at the G_1-S phase interface, which has been evaluated in several phase II clinical trials with activites against some prostate and pancreatic cancers [43-44].

Recent progress on the biosynthesis pathway of illudins

The biosynthesis of illudins begins with cyclization and rearrangement of universal precursor farnesyl diphosphate (FPP) catalyzed by protoilludene synthase, to yield a sesquiterpenoid protoilludane. Protoilludane can be further modified by accessory enzymes such as cytochrome P450 monoxygenases and other types of oxidoreductases, obtaining the final illudin product [45].

Several protoilludene synthases from Basidiomycetes have been reported. The first protoilludene synthase, designated as *Pro1*, was identified from *Armillaria gallica* [46]. Further analysis of *Pro1* revealed an open reading frame 1041 bp in length encoding a protein with 347 amino acid residues and a predicted molecular mass of 40 kDa. Heterologous expression of *Pro1* in *E. coli* resulted in one peak, suggesting that it is also a highly specific enzyme, although the activity of this enzyme has not been fully studied *in vitro* [46]. Then, Wawrzyn and co-workers reported two protoilludene synthases Omp6 and Omp7 from Jack O'Lantern mushroom *Omphalotus olearius*, which was first identified for illudins S, and Omp6 and Omp7 were highly active and product-specific protoilludene synthases, producing 6-protoilludene *via* a likely 1, 11 ring closure of FPP to create a trans-humulyl cation intermediately, followed by two cyclization steps to yield the final volatile sesquiterpene [47]. The crystallization and preliminary X-ray analysis of the 357-amino-acid 41 068 Da Omp6 protoilludene synthase were also reported [48]. In 2013, three 1, 11-cyclizing protoilludene synthases, named as Stehi1|64702, Stehi1|73029, and Stehi1|25180, from *Stereum hirsutum* were successfully predicted and cloned, while their functions were confirmed [21]. According to i*n vitro* analysis, there was a small amount of *β*-elemene in the headspace of Stehi1|64702 and Stehi1|73029 expressing cultures, suggesting that these protoilludene synthases are less specific than Stehi1|25180, with only one single peak by GC/MS [21]. Based on the bioinformatic survey of BLAST programme and known STSs on the genomic database of *Postia placenta*, Ichinose *et al*. found that PpSTS-08 and PpSTS-18 were shown to synthesize Δ^6-protoilludene as a single major product by GC-MS analysis, although the kinetic parameters of these enzymes have not been fully studied [49]. Very recently, Zhang *et al*. searched Agr6 and Agr7 as protoilludene synthases through BLASTP search in the genome of *Agrocybe aegerita*, which is a prominent fungal species with good application value and solid fundamental background [50]. Furthermore, the following three sesquiterpene synthases, Denbi1_659367 from *Dendrothele bispora*, Hetan2_454193 from *Heterobasidion annosum*, and Hypsu1_138665 from *Hypholoma sublateritium*, which show a close relationship to Agr6 and Agr7, were cloned and verified as protoilludene synthases [50].

P450s and/or other enzymes from mushroom-forming fungi (Basidiomycetes) responsible for the biosynthesis of illudins remain poorly understood. P450 6i, located in the same

cluster of OMP6, was the only proved P450 for the biosynthesis of illudins [51]. The monooxygenase activity of P450 6i to modify protoilludene was examined in *E. coli*, *S. cerevisiae* and *P. pastoris*, respectively. The identified enzymes involved in the biosynthetic pathway of illudins are shown in Fig. 4B and listed in Table 1.

Synthetic biology intervention on illudins

Protoilludane, the precursor of illudins, was produced in *E. coli* using synthetic biological methods. Protoilludene was first generated in *E. coli* through overexpression of a hybrid exogenous MVA pathway, endogenous FPP synthase (IspA), and protoilludene synthase (OMP7) from *O. olearius*. Through sequential order permutation of the lower MVA pathway, the alteration of promoters and copy numbers for the upper MVA pathway, the coordination of the lower and upper portions, and homolog substitution with the corresponding genes, protoilludane production finally reached to 1199 mg·L^{-1} [52]. In 2017, the same group produced protoilludene *via* the 2C-methyl-D-erythritol 4-phosphate (MEP) pathway, the native pathway in *E. coli*. By overexpression of the Fpr and FldA protein complex, which mediated electron transfer from NADPH to Fe–S cluster proteins; by overexpression of NADH kinase tPos5p that converted NADH to NADPH; and by deletion of a promiscuous NADPH-dependent aldehyde reductase (YjgB) that consumed NADPH to increase the supply of NADPH, the productionof protoilludene was increased to 512.7 mg·L^{-1} [53]. An overview of the strategies for improving protoilludene production in *E. coli* is shown in Fig. 4C.

Lagopodins

Lagopodins is a type of quinone sesquiterpenes with antimicrobial activity [17]. The biosynthetic pathway of lagopodin A was first identified in *Coprinopsis cinerea*, commonly referred to as the grey shag mushroom and the sole model Basidiomycete for investigating fruiting body development, mating, and evolution in mushrooms [54]. Generated from the common precursor of sesquiterpene, FPP was catalyzed to *α*-cuprenene by Cop6 [17], which was identified based on interpretation of the whole genome sequencing data of *C. cinereal* [55]. Then, based on the *ku70*-deficient strain of *C. cinereal*, two P450s, Cox1 and Cox2 which participated in the biosynthetic pathway of lagopodin A, were identified [56]. However, kinetic characterization studies did not carried out due to the difficulty in preparing sufficient amounts of substrates and purified P450s [56] (Fig. 5A).

α-Cuprenene, the direct precursor of lagopodin A, was successfully produced in *E. coli*, *S. cerevisiae* and *X. dendrorhous* [57]. *α*-Cuprenene was produced by the *E. coli* strains transformed with the Cop6 gene through addition of a dodecane organic phase to liquid cultures [58], and the production reached to approximately 0.25 mg·L^{-1} after 48 h. By virtue of constitutive expression of Cop6 gene in *S. cerevisiae*, the production of *α*-cuprenene reached to 6.6 mg·L^{-1} on the second day of culture. In *X. dendrorhous*, *α*-cuprenene was produced *via* Cop6 gene integrated in the rDNA of the yeast, inserted in the *crtE* gene or *crtYB* gene and caused the disruption of carotenoid production in rich and minimal media. The

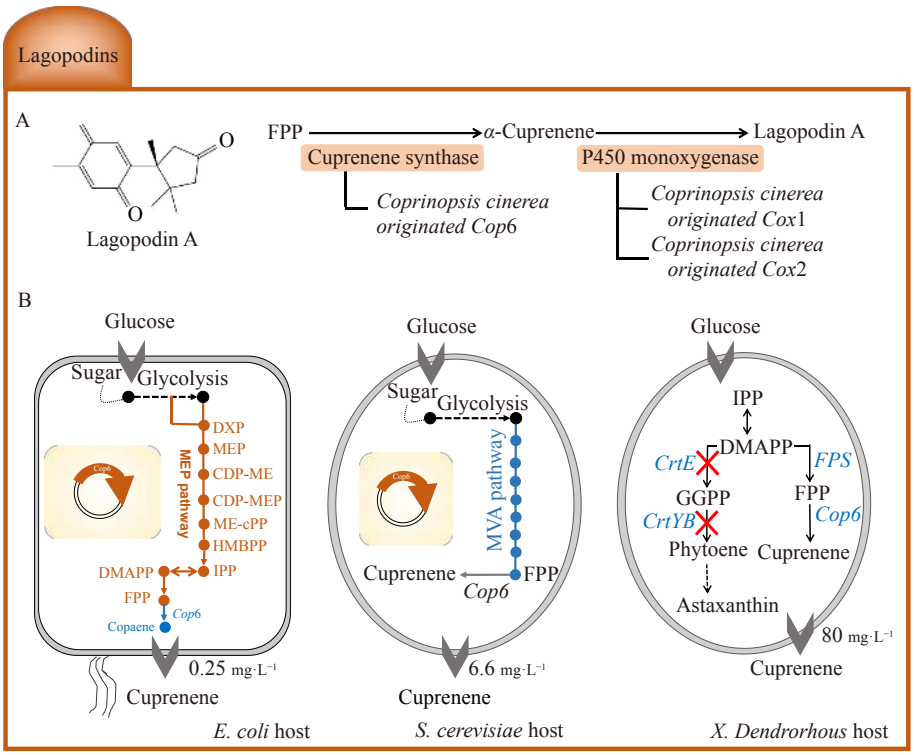

Fig. 5 Diagram of lagopodin A biosynthesis pathway and *E. coli*, *S. cerevisiae* and *X. dendrorhous* as the production hosts for the intermediate cuprenene production

highest yield was almost 80 mg·L^{-1} in YPD rich medium from a strain harboring Cop6 gene integrated in the rDNA after culture for 96 h; while the maximum production of α-cuprenene was 20 mg·L^{-1} in minimal medium from s strain harboring Cop6 inserted in *crtYB* gene (Fig. 5B).

Psilocybin

The indole alkaloid psilocybin (4-phosphoryloxy-*N*, *N*-dimethyltryptamine) is the principal natural product of hallucinogenic *Psilocybe* mushrooms [59]. Psilocybin, originally purified from *Psilocybe mexicana* mushroom by Hofmann in 1958 [60], has been demonstrated for treatmenting anxiety in terminal cancer patients [61, 62] and alleviating the symptoms of post-traumatic stress disorder [63]. The biosynthetic pathway of psilocybin was characterized by Fricke *et al*. [64]. Psilocybin biosynthetic gene clusters (psi) have been identified from *Psilocybe cubensis* and *Psilocybe cyanescens*. Four psilocybin biosynthesis enzymes were characterized, including a new class of fungal L-tryptophan decarboxylases (PsiD), a kinase (PsiK), a *S*-adenosyl-L-methionine (SAM)-dependent *N*-methyltransferase (PsiM), and a P450 monooxygenase (PsiH). L-Tryptophan was first decarboxylated to give tryptamine with PsiD, which belongs to the PLP-independent phosphatidylserine decarboxylase family (E.C. 4.1.1.65). Interestingly, PsiD also accepted 4-hydroxy-L-tryptophan as a substrate for decarboxylation. Subsequent modification reactions were mediated by PsiH, PsiK and PsiM in a virtually linear process to biosynthesize psilocybin [64] (Fig. 6A).

The Hoffmeister group produced psilocybin *in vivo* using an eukaryotic host, *Aspergillus nidulans*, at titers reported near 100 mg·L^{-1} [65]. Utilizing the genes of PsiD, PsiK, and PsiM from *P. cubensis*, together with the promiscuity of the native *E. coli* tryptophan synthase TrpAB, Adams *et al*. produced psilocybin from 4-hydroxyindole in model organism *E. coli* BL21 star™ (DE3) [66]. Psilocybin production was then optimized through the methods including: (1) a defined three-level copy number library, (2) a random 5-member operon library, and (3) a random 125-member pseudooperon library. After transcriptional optimization and fermentation condition optimization, the highest psilocybin production was at about 139 ± 2.7 mg·L^{-1} (Fig. 6B).

Viridiflorol

Viridiflorol exhibits multiple biological activities such as antibacterial, anti-inflammatory and antioxidant activities, which can be potentially applied in agricultural and food products to replace current broad-spectrum toxic pesticides and unhealthy food preservatives [67-69]. In 2016, from the genome of the black poplar mushroom, *Agrocybe aegerita*, two novel viridiflorol synthase (VS), Agr2 and Agr5 were first characterized. Based on this identification, in 2020, *Sphaerobolus stellatus* originated Sphst_47084 and *Dendrothele bispora* originated Denbi1_816208 were selected and experimentally validated as viridiflorol synthases by the same group [50]. For the biosynthesis of viridiflorol, Shukal *et al*. engineered *E. coli* cells to produce viridiflorol directly from sugar by virtue of Agr5 [70]. They selected modular pathway design and promoters of different strengths to balance the pathway at the transcriptional dimension; used regression models to quantitatively describe the reliance of metabolite production on inducer concentrations by detailed titration; designed two ribosomal binding site (RBS) libraries for VS that

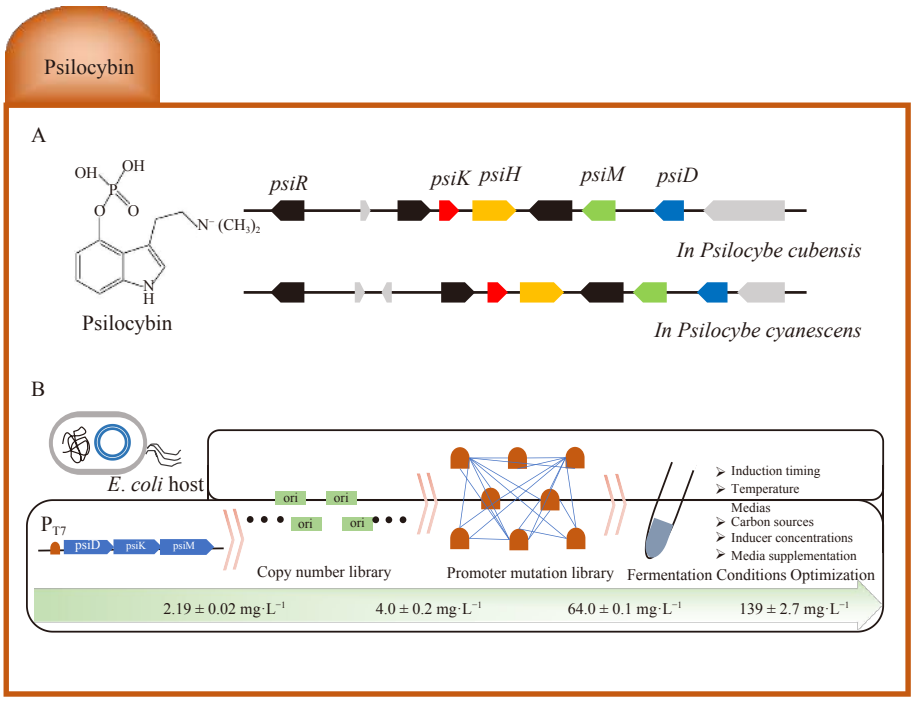

Fig. 6 Diagram of psilocybin biosynthesis pathway and *E. coli* as the production host for psilocybin production

covered a broad range of translational initiation rates (TIRs) from 4000 to 18 206 a.u.; introduced random mutations to VS by error-prone PCR; and created an auxotrophic *E. coli* strain by deleting three aromatic-amino-acid synthesis genes from the genome. Finally, the engineered *E. coli* strain produced 1.4 g·L^{-1} viridiflorol of dry cells from 10 g·L^{-1} glucose (Fig. 7).

Research Progress on the Isoprene Building Blocks in Mushrooms

All terpenoids are based on the same basic C5 isoprene building blocks, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), which are consecutively fused by head to tail condensation. Depending on their carbon chain length, these linear phosphorylated alkenes are universal precursors of mono(C10)-, sesqui(C15)-, di(C20)-, ester(C25)- or tri(C30)-terpenes [71]. FPP, a common precursor for terpenoids, is synthesized by the condensation of three 5-carbon isoprenyl pyrophosphate units that are derived from acetyl-coenzyme A (acetyl-CoA) through the common mevalonate (MVA) pathway and non-mevalonate (MEP) pathway in mushrooms. By transcriptome analysis of *Polyporus brumalis*, the genes encoding HMG-CoA synthase (EC 2.3.3.10), acetyl-CoA C-acetyltransferase (EC 2.3.1.9), diphosphomevalonate decarboxylase (EC 4.1.1.33) and isopentenyl-diphosphate isomerase (EC 5.3.3.2), which are involved in the MVA pathway, were identified [72]. Meanwhile, the genes involved in the MEP pathway, including 1-deoxy-d-xylulose-5-phosphate synthase (EC 2.2.1.7), 1-deoxy-d-xylulose-5-phosphate reductoisomerase (EC 1.1.1.267), 2-C-metyl-d-erythriol 2, 4-cyclodiphosphate synthase (EC 4.6.1.12), 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase (EC 1.17.7.1) and 4-hydroxy-3-methylbut-2-enyl diphosphate reductase (EC 1.17.7.2) were also identified [72]. In *Stereum hirsutum*, Flynn *et al*. identified a new type of fusion protein between an STS (HS) and an HMG-CoA synthase (HMGS) domain, referred to here as HS-HMGS, which catalyzed the second reaction in the MVA pathway and cyclization of FPP reaction [73].

Conclusions and Perspective

Mushrooms are an exellent source of natural active compounds and up to now, some natural active compounds have been discovered. However, the contents of these natural compounds in mushrooms are too low to meet the increasing demand for medical use, and large-scale production by chemical synthesis is not practical at present due to the complexity of natural compounds and the environmental pollution of chemical synthesis. These issues seriously limit further pharmacological research and new drug development from mushrooms. In the current study, we summarize the recent progress on the biosynthetic pathways and key enzymes involved in the biosynthesis of mushroom originated natural compounds, so as to lay a foundation for improving the yield of natural compounds in mushrooms through regulation of the biosynthetic pathways and provide building blocks for natural compound production by synthetic biological methods.

Many mushroom strains are difficult to grow under laboratory conditions, requiring suitable growth conditions and long fermentation time. Furthermore, there is a lack of genetic tools and techniques available for the transformation and genetic manipulation of mushrooms. Therefore, characterization of their biosynthetic genes typically requires heterologous expression in a more genetically tractable host. Production of mushroom originated natural compounds by metabolic engineering is an attractive strategy to improve the yield and much attention has focused on related biosynthetic enzymes. In recent years, great progress has been

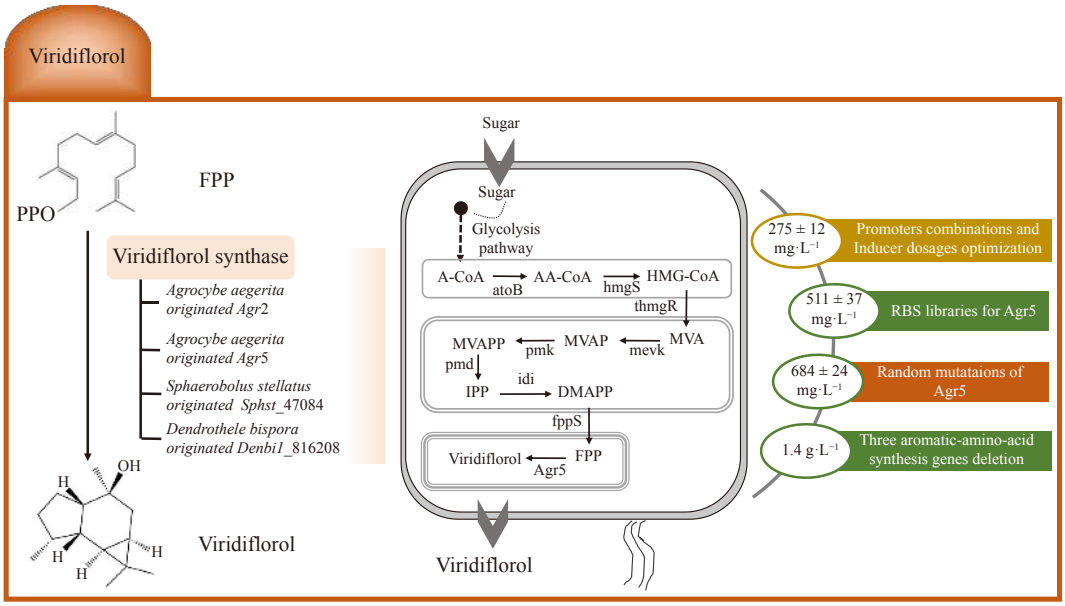

Fig. 7 Diagram of viridiflorol biosynthesis pathway and *E. coli* as the production host for viridiflorol production

achieved in studies concerning the metabolic engineering of mushroom originated natural compounds in microbes. However, there are still limits for the production of mushroom originated natural compounds in heterologous microbes. From a macro perspective, engineered microbes may pose potential hazards to biosafety and environment safety. From a technical perspective, *E. coli*, the most common host for biosynthesis, appears imperfect for the expression of genes derived from eukaryotic donors, where post-translational modifications are not required for full catalytic activity. From an implementation perspective, there is a long way for this type of fundamental research to mass production for market demand. These issues are the urgent challenges for synthetic biologists in the coming future.

In general, microbes as the production host, provides an alternative and attractive approach for the production of mushroom originated natural compounds. This review summarizes the latest research progress on the biosynthetic pathways of mushroom originated natural active compounds and synthetic biology intervention on the synthesis of natural active compounds, which lays a foundation for the screening of new natural active compounds from mushrooms, provides new thoughts on mushroom studies, and improves the production of other active compounds by virtue of microbes.

Abbreviations

*γ*GC, *γ*-glutamylcysteine; *γ*GC-HER, hercynyl-*γ*-glutamylcysteine sulfoxide; A-CoA, acetyl-CoA; AA-CoA, acetoacetyl-CoA; CDP-ME, 4-diphosphocytidyl-2-C-methyl-D-erythritol; CDP-ME2P, 2-phospho-4-diphosphocytidyl-2-C-methyl-D-erythritol; Cys-HER, hercynylcysteine sulfoxide; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; ERG, ergothioneine; FPP, farnesyl pyrophosphate; G-3-P, glyceraldehyde 3-phosphate; HMBPP, 1-hydroxy-2-methyl-2-(*E*)-bttenyl-4-diphosphate; HER, hercynine; HMG-CoA, hydroxymethylglutary-CoA; IPP, isopentenyl diphosphate; L-His, L-histidine; MECP, 2-*C*-methyl-D-erythritol-2, 4-cyclodiphosphate; MEP, 2-*C*-methyl-D-erythritol-4-phosphate; MVA, mevalonate; MVAP, mevalonate 5-phosphate; MVAPP, mevalonate diphosphate; Dxs, 1-deoxy-D-xylulose-5-phosphate synthase; IspC, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; IspD, 4-diphosphocytidyl-2-*C*-methyl-D-erythritol synthase; IspE, 4-diphosphocytidyl-2-*C*-methylerythritol kinase; IspF, 2-*C*-methyl-D-erythritol-2, 4-cyclodiphosphate synthase; IspG, 4-hydroxy-3-methylbut-2-enyl-diphosphate synthase; IspH, 1-hydroxy-2-methyl-butenyl-4-diphosphate reductase; MvaE, bifunctional acetoacetyl-CoA thiolase and HMG-CoA reductase; MvaS, HMG-CoA synthase; MvaK1, mevalonate kinase; MvaK2, phosphomevalonate kinase; MvaD, mevalonate diphosphate decarboxylase; IDI, IPP isomerase; ispA, FPP synthase.

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