

REVIEW

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Advances in the sustainable biosynthesis of valuable terpenoid flavor compounds and precursors in micro-organisms

Qian Li¹, Yibo Ning², Deyu Liu¹, Jie Ren¹, Changtong Lu², Zhifei Chen^{2*} and Guanglu Wang^{1*}

Abstract

Renowned for their distinctive aromas, terpenoid flavor compounds and their precursors are widely used in medicine, food, and the flavor and fragrance industries. Rapid advances in synthetic biology, including the modification of microbial chassis cells, the design of synthetic pathways for novel target products, and the integration of large-scale microbial fermentation, have enabled the development of microbial cell factories for the green and efficient production of terpenoid flavor compounds and their precursors, offering broader market potential. This review examines common biosynthetic mechanisms, recent progress in the field, and strategies for enhancing the biosynthetic efficiency of terpenoid flavor compounds and their precursors. This study aims to support the advancements of sustainable production technologies and promote industrial application within the flavor and fragrance sector.

Keywords Terpenoids, Flavors and fragrances, Microbial cell factory, Biosynthetic strategy

Introduction

Terpenoids are a class of compounds formed through repetitive condensation reactions between isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) (Fig. 1). Based on their isoprene unit count, they are classified as monoterpenes (C₁₀), sesquiterpenes (C₁₅), diterpenes (C₂₀), triterpenes (C₃₀), tetraterpenes (C₄₀), and polyterpenes [1, 2]. These compounds are widely distributed in nature, exhibiting diverse biological activities and aromatic properties; hence, they are valuable in various applications, such as food and cosmetic fragrances [3]. Terpene aroma compounds, including

nerol, linalool, and borneol, contribute distinct scents to fragrances [4, 5]. Precursor compounds undergo chemical transformations via processes such as high-temperature pyrolysis, thereby yielding aromatic components, such as abietadiene, sabinene, and ambergris alcohols.

With evolving consumer preferences, the demand for natural terpenes is increasing. Currently, they are primarily obtained from plant extracts and chemical synthesis. However, these conventional methods face challenges, including low raw material content, high extraction costs, and environmental concerns, which limit their ability to meet market demand. In contrast, microbial biocatalysis offers considerable potential advantages. First, it is a streamlined technological process that significantly shortens the production cycle, thereby enhancing its cost-effectiveness and competitiveness in future markets. Second, it demonstrates superior environmental sustainability, closely aligning with the principles of green production [6–8].

Moreover, according to the flavoring regulations established by the U.S. FDA and the European Union (EC No.

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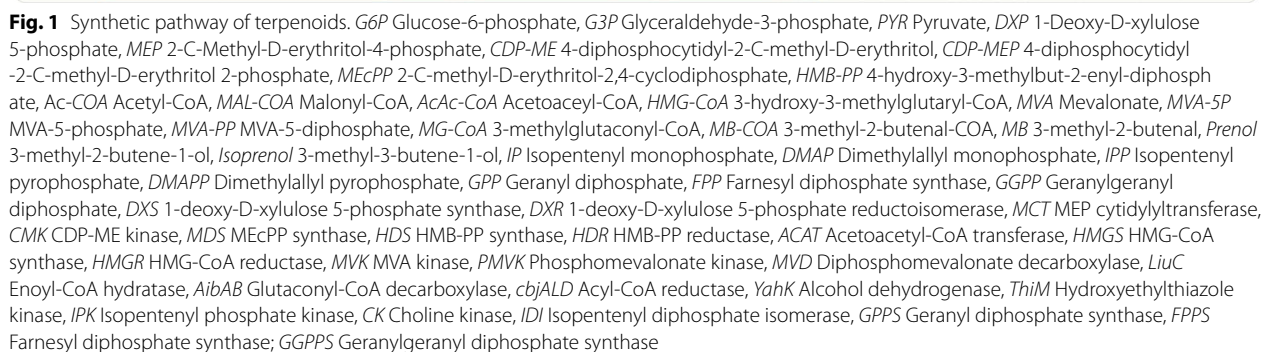
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strain and had a significant impact on reducing production costs [10]. However, traditional production methods for most terpenoid flavor compounds still offer a cost advantage, and biosynthetic approaches have not yet fully replaced conventional routes. Techno-economic analyses of microbial terpenoid production have consistently shown that productivity (i.e., production rate) and yield (i.e., total output) are the primary determinants of production cost. Thus, increasing product concentration is essential for achieving commercial viability [11, 12].

Researchers have used various hosts, including *Escherichia coli*, *S. cerevisiae*, and *Yarrowia lipolytica*, as chassis organisms to construct efficient microbial cell factories for ecofriendly terpenoid synthesis [13–16]. *E. coli* is a widely used bioengineering micro-organism. It offers advantages, including facile genetic modification, rapid growth, and high-density cultivation, making it particularly useful for synthesizing small-molecule terpenoids, such as linalool and limonene [17]. However, the

biosynthesis of oxygenated terpenoids such as nootkatone is strictly dependent on the activity of cytochrome P450 (CYP) enzymes. These enzymes act as rate-limiting catalysts in terpenoid pathways and face inherent obstacles for efficient functional expression in prokaryotic systems [18]. In contrast, yeast systems (e.g., *S. cerevisiae*) provide a robust cellular milieu for P450 activity through eukaryotic organelles (e.g., endoplasmic reticulum), dedicated chaperone networks, and endogenous redox partner systems that facilitate proper folding, post-translational modification, membrane integration, and catalytic functionality [19, 20]. Therefore, yeast is the preferred chassis for terpenoid biosynthesis involving P450-dependent reactions. Overall, selecting an appropriate host organism is important for efficient terpenoid biosynthesis [21].

This review examines recent advances in the biosynthesis of key terpenoid flavor compounds and their precursors using *E. coli* and yeast as chassis organisms. This study also summarizes methodologies and strategies to enhance biosynthetic efficiency and promote green synthesis routes for terpenoid flavor compounds and their precursors, thereby supporting their future application and competitiveness within the flavor and fragrance industry.

Engineering *E. coli* for terpenoid flavor compound and precursor production

Escherichia coli offers several advantages for terpenoid synthesis. Its growth cycle is markedly shorter than that of eukaryotes, including yeast, allowing it to rapidly reach high cell density and improve synthesis efficiency [22]. Furthermore, *E. coli* is highly amenable to genetic modification. Numerous metabolic engineering tools, including plasmids, promoters, and regulatory elements, have been developed for *E. coli* to regulate key enzymes in terpenoid synthesis pathways [23]. Its high carbon utilization efficiency, simple fermentation requirements, and cost-effective cultivation further enhance the viability of *E. coli* as a host for terpenoid biosynthesis [24, 25].

In this section, we summarize the yields of terpenoid flavor compounds and their precursors synthesized through *E. coli* recently (Table 1). Notably, the biosynthesis of several compounds has reached levels suitable for industrial applications. Overall, *E. coli* has considerable potential for terpenoid biosynthesis through genetic engineering and metabolic pathway optimization.

Terpenoid flavor compounds

Nerol

Nerol is a monoterpene with a floral fragrance and a complex synthetic pathway. Recent studies have focused on optimizing substrate carbon flux regulation and

improving the catalytic efficiency of nerol synthase (*GmNES*) to enhance yield and efficiency. Zong et al. used glucose as the carbon source and co-expressed truncated neryl diphosphate synthase and *GmNES* to achieve a nerol yield of 0.053 mg/L [26]. By overexpressing genes in the mevalonate (MVA) pathway to boost IPP and DMAPP supply and increasing carbon flux toward nerol synthesis via *ERG10* overexpression, they achieved a ~30-fold yield increase, reaching 1.6 mg/L in shake flask cultures. Lei et al. engineered *E. coli* using metabolic and protein engineering strategies for de novo nerol synthesis [27]. They screened 11 endogenous and heterologous phosphatases and replaced *GmNES*, finding that the endogenous hydrolysis enzyme NudJ in *E. coli* produced a higher nerol yield (261.88 mg/L). Shake flask fermentation further increased the yield to 966.55 mg/L.

Geraniol

Geraniol, a key fragrance component, is widely used in the food, cosmetics, and perfume industries. Its biosynthetic pathway involves two essential enzymes: geranyl diphosphate synthase (GPPS) and geraniol synthase (GES). Recent research has substantially improved geraniol synthesis through metabolic pathway engineering. Liu et al. used GPPS from *Abies grandis* and GES from *Ocimum basilicum* to synthesize geraniol in *E. coli* via the MVA pathway [28]. The addition of isopropyl myristate reduced geraniol volatilization and inhibited its conversion to geranyl acetate. Their study also revealed that *E. coli* acetyltransferase converts geranyl acetate to geraniol, increasing the yield to 2.0 g/L through acetyltransferase overexpression and starvation strategies. Wang et al. screened GPPS and GES, developed an *E. coli* platform strain for geraniol production, and markedly improved its yield [29]. Using fusion tag evolution engineering to optimize GES expression and increase metabolic flux from GPP to geraniol, they achieved a geraniol titer of 2124.1 mg/L through shake flask fermentation. Shukal et al. identified a novel enzyme *RhNUDX1*, derived from roses as an alternative to conventional GES. This approach effectively circumvented the limitations associated with plant-derived enzymes, such as protein precipitation and low catalytic activity, and achieved a geraniol titer of 0.91 g/L in shake flasks. This study proposes a novel alternative biosynthetic pathway for geraniol [30].

Limonene

Limonene, a monoterpene with a citrus aroma, is widely used in fragrances, cleaning products, and pharmaceuticals. Its biosynthesis requires multiple enzymes, particularly limonene synthetase (LS). Enhancing LS expression through genetic engineering is key to increasing limonene production. In addition, using nontoxic

Table 1 Synthesis levels and aroma profiles of terpenoid flavor compounds and fragrance precursors in *E. coli*

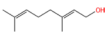
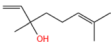
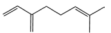
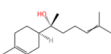
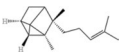
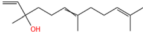
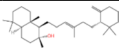
Product	Chemical formula	Titers (g·L ⁻¹)	Scale	Productivity (g·L ⁻¹ ·h ⁻¹)	Aroma description	References
Nerol		0.97	shake flask	0.012	Fresh, sweet orange blossom and rose fragrance, with fruity notes reminiscent of raspberries; sweet with a hint of bitterness	[27]
Geraniol		2.12	shake flask	0.044	Resembling the scent of roses; a lingering fragrance with a subtle bitterness	[29]
Limonene		7.30	3.1-L fed batch	0.15	Fresh tangerine-lemon fruit scent with sweet green acidity; a light aroma	[33]
Farnesol		0.53	shake flask	0.011	Warm floral scent, complemented by a subtle sweetness and a hint of fruitiness	[48]
Linalool		4.16	2-L fed batch	0.035	Lily of the valley scent, with lilac and rose flowers; a soft, light, lasting aroma	[51]
α-Pinene		1.26	1.5 mL eppendorf tubes	0.10	Pure piney, woody and turpentine-like aroma	[42]
Borneol		87.80 mg·L ⁻¹	shake flask	0.77 mg·L ⁻¹ ·h ⁻¹	Intense fresh camphor aroma, with subtle woody and herbal undertones	[27]
(R)-(+)-Perillyl alcohol		0.45	5-L fed batch	1.71 mg·L ⁻¹ ·h ⁻¹	Fresh, subtly sweet herbal aroma, complemented by the cool, distinctive fragrances of perilla leaves and mint	[150]
Myrcene		1.25	–	0.014	Delightful light balsamic aroma, sweet, fruity and fresh citrus notes	[151]
(–)-α-Bisabolol		23.40	1-L fed batch	0.24	Soft floral notes similar to chamomile or freesia, light floral	[152]
τ-Cadinol		133.50 mg·L ⁻¹	shake flask	0.0037	Rich woody notes, complemented by an earthy aroma, with warm herbs, and a subtle spicy undertone	[153]
α-Santalene		2.92	1.3-L fed batch	0.48 mg·L ⁻¹ ·h ⁻¹	Sweet, mild, and woody fragrance, a pleasant and long-lasting aroma	[77]
Nerolidol		16.00	5-L fed batch	0.17	Subtle floral fragrance, reminiscent of roses and apples, characterized by sweetness, freshness, and longevity	[47]
β-Elemene		3.52	4-L fed batch	–	Spicy and woody scent	[154]
Patchoulol		0.97	5-L fed batch	0.0083	Intense resinous aroma and herbal fragrance with minty, woody base notes	[155]
β-Caryophyllene		5.14	2-L fed batch	0.080	Distinctive spicy, woody, and clove-like aroma	[84]
Geranylinalool		2.06	5-L fed batch	0.014	Sweet and fresh floral aroma, similar to lily of the valley	[54]
cis-Abienol		1.38	1.3-L fed batch	0.012	Oxidative degradation produces amber-like compounds with a unique fragrance	[58]

Table 1 (continued)

Product	Chemical formula	Titers (g·L ⁻¹)	Scale	Productivity (g·L ⁻¹ ·h ⁻¹)	Aroma description	References
Ambrein		2.60 mg·L ⁻¹	shake flask	0.054 mg·L ⁻¹ ·h ⁻¹	Oxidation and degradation form aromatic compounds, e.g., ambroxide, with a floral and vegetable oil-like fragrance	[62]

organic solvents to mitigate limonene's inhibitory effects on micro-organisms and optimizing the MVA pathway are crucial strategies for boosting biosynthetic yield [31]. Willrodt et al. heterologously introduced the *S. cerevisiae* MVA pathway into *E. coli* using GPPS from *A. grandis*, and optimized and truncated LS codons from *Mentha spicata* [32]. Using glycerol as the sole carbon source, they obtained 2.7 g/L limonene in a 3.1-L fermenter. Rolf et al. optimized MVA pathway-related genes and LS from *Mentha haplocalyx* in *E. coli*, producing strain pJBEI-6410 [33]. During fermentation, they added a non-toxic organic solvent, diisononyl phthalate, to a 3.1-L fermenter. Using glycerol as the sole carbon source, they achieved a final limonene yield of 3.6 g/L.

α -Pinene

α -Pinene is a prevalent bicyclic monoterpene with notable anti-inflammatory and neuroprotective properties. It is synthesized via the MVA or MEP pathway, resulting in the formation of GPP, which is subsequently cyclized by α -pinene synthase (PS) [34, 35]. Bokinsky et al. first demonstrated microbial α -pinene production in *E. coli* by heterologously expressing the MVA pathway, GPPS, and PS, achieving a titer of 1.7 mg/L [36]. Subsequently, Yang et al. engineered *E. coli* strain YJM28 with a hybrid MVA pathway, GPPS2, and PS (Pt30), yielding a titer of 0.97 g/L under fed-batch fermentation conditions [37]. Further enhancements included the construction of GPPS–PS fusion proteins to ameliorate GPP feedback inhibition using computational enzyme engineering techniques, such as truncations and site-directed mutagenesis, and the development of modular *E. coli* coculture systems to distribute the metabolic burden [38–41]. The highest reported α -pinene titer to date, 1.26 g/L, was obtained using a cell-free biosynthesis system optimized for lysate composition and enzyme ratios [42]. However, α -pinene exhibits significant toxicity toward *E. coli*, and the limitations in precursor supply efficiency and the stability of the MVA/MEP pathways are challenges for large-scale production [43]. Future efforts should integrate host tolerance engineering and advanced enzyme evolution techniques to achieve sustainable and industrial-scale biosynthesis of α -Pinene [35].

Nerolidol

Nerolidol, a sesquiterpene compound, emits distinct floral and woody scents. It has potent antioxidant and antibacterial properties. Thus, it is widely used in cosmetics, personal care products, and other commercial products [44–46]. Tan et al. engineered *E. coli* as a chassis organism to progressively enhance its synthetic yield through a systematic engineering approach [47]. They initially screened seven distinct nerolidol synthase (LNS) enzymes from different sources and ultimately identified *Fa*LNS from strawberry as the most catalytically active variant. Subsequently, promoter engineering was used to regulate the expression of the MVA pathway modules, knocked out byproduct synthesis genes (Δ *ldhA*, Δ *pflB*, etc.) to block competing pathways, and optimized precursor supply through a glucose–glycerol–lactose mixed carbon source to achieve a nerolidol yield of 3.3 g/L in shake flasks (carbon conversion rate: 26.2%, >90% of theoretical yield). Finally, using a two-phase extraction fed-batch fermentation process (with the addition of dodecane as the organic phase), 16 g/L of nerolidol was obtained in a 5-L bioreactor (carbon yield of 9%). This study established a new record for the highest microbial production of nerolidol, and its efficient carbon conversion strategy provides a foundation for low-cost industrial production.

Farnesol

Farnesol, a sesquiterpene alcohol, has considerable industrial value owing to its applications in perfumes, medicines, and biofuels. In micro-organisms, farnesyl diphosphate (FPP) can be hydrolyzed using phosphatase enzymes to produce farnesol. Wang et al. first heterologously introduced the MVA pathway and screened phosphatase genes upregulated during FPP accumulation [48]. The phosphatases YbjG and PgpB exhibited substantial FPP hydrolysis activity. In subsequent experiments, they demonstrated that overexpressing *ispA*–PgpB or *ispA*–YbjG markedly increased farnesol production. Using the engineered strain, they achieved a farnesol yield of 526.1 mg/L after 48 h of shake flask fermentation, which was 7.1-fold higher than that in the control group.

Linalool

Linalool, a floral monoterpene compound, is produced via linalool synthase (LIS). Improving LIS catalytic efficiency or optimizing its expression levels are key strategies for increasing linalool production [49]. Wu et al. used *ScLIS* from *Streptomyces clavuligerus* and enhanced its expression by modifying the ribosome binding site (RBS) [50]. They then used protein scaffolds to spatially colocalize *EcIDI*, *AgGPPS*, and *ScLIS*, thereby improving the catalytic efficiency of the cascade. By optimizing the fermentation conditions, a linalool yield of 1523 mg/L was achieved. Linalool production can be further enhanced by increasing acetyl-CoA supply, reducing byproduct accumulation, and improving substrate utilization through metabolic engineering. Wang et al. introduced the MVA pathway into *E. coli*, coupled with GPPS and *MaLIS* from *Mentha aquatica*. They optimized RBS and performed site-specific mutations to boost linalool synthesis. To minimize pyruvate loss and acetate accumulation, an engineered NADP⁺-dependent pyruvate dehydrogenase complex and a phosphoketolase bypass pathway were constructed, yielding 4160 mg/L of linalool [51].

Nootkatone

Nootkatone, a natural sesquiterpene with a citrus aroma, is widely used in flavorings and health supplements. Valencene synthase from *Citrus sinensis* can be efficiently expressed in *E. coli*, facilitating its use in nootkatone synthesis [52]. Girhard et al. screened 120 cytochrome P450 enzymes from various bacterial sources and selected *E. coli* as the chassis organism for the coexpression of CYP109B1 from *Bacillus subtilis*, putidaredoxin from *Pseudomonas putida*, and its reductase PdR. Consequently, they achieved a nootkatone yield of 120 mg/L [53].

Geranyllinalool

Geranyllinalool, a sesquiterpenoid compound synthesized from GPP and linalool, possesses a distinctive rose-like fragrance. Chang et al. engineered an artificial isoprenol utilization pathway (IUP) in *E. coli* to convert isoprenol into geranyllinalool via a two-step enzymatic process [54]. They screened geranyllinalool synthases from various sources and identified the enzyme from *Nicotiana attenuata* as the most efficient, achieving a yield of 274.78 mg/L geranyllinalool. Subsequently, they systematically investigated the effects of carbon source, isopropyl β -D-1-thiogalactopyranoside concentration, and isoprenol addition on product yield. Based on these findings, they scaled-up production in a 5-L fermenter. They achieved a final titer of 2.06 g/L geranyllinalool through fed-batch fermentation.

Sclareol

Sclareol is a natural diterpenoid primarily isolated from *Salvia sclarea* and is the major constituent of its essential oil. Schalk et al. used a functional genomics strategy to identify and characterize two previously unknown diterpene synthases, *SsLPS* and *SsScS*, from a sage flower cDNA library [55]. To establish a microbial biosynthesis process for sclareol, they introduced the MVA pathway into *E. coli* and overexpressed *ERG20* to increase intracellular FPP levels, thereby increasing GGPP availability, which is a key precursor for diterpene synthesis. By coexpressing the *SsLPS* and *SsScS* enzymes, a yield of 418 mg/L was achieved in shake flask cultures. Moreover, when a two-phase fed-batch fermentation system containing dodecane as the organic phase was implemented in a 5-L bioreactor, the final titer of sclareol reached 1.5 g/L. This study provided the first experimental evidence for the dual-enzyme biosynthetic mechanism responsible for sclareol production in sage, laying the foundation for its industrial-scale microbial synthesis.

Flavor precursors

Cis-abienol

Cis-abienol is a natural diterpenoid that is predominantly found in the essential oils of pine and fir. Research on *cis-abienol* biosynthesis has focused on optimizing metabolic pathways and identifying key enzymes, such as *cis-abienol* synthase and *abienol* synthase [56]. Li et al. engineered the 1-deoxy-D-xylulose 5-phosphate (DXP) and MVA pathways in *E. coli*, which markedly increased *cis-abienol* production [57]. Screening results showed that *cis-abienol* synthase from *Abies balsamea* and *abienol* synthase from *Salvia sclarea* exhibited high catalytic activity. Using isopropyl myristate as a solvent in a fed-batch bioreactor, the final *cis-abienol* yield reached 634.7 mg/L. Zhang et al. introduced IUP into *E. coli* to enhance DMAPP supply by screening and optimizing ethanol kinases and isoprenyl phosphate kinases [58]. They further knocked out the *aphA* and *yqaB* genes to construct strain BD203 with double phosphatase deletion, further increasing DMAPP accumulation. After 112 h of fermentation in a 1.3-L fermenter, the engineered strain produced 1375.7 mg/L of *cis-abienol*.

Cembratriene-diol

Cembratriene-diol (CBT-diol) is a diterpenoid that is degraded into solanone, norsolanedione, and solanifuran. It is initially synthesized as *cembratrien-ol* (CBT-ol) from GGPP via *cembratrien-ol* synthase (CBTS) and is then oxidized by cytochrome P450 hydroxylase [59]. Schrepfer et al. introduced *Nicotiana tabacum* CBTS gene into *E. coli* chassis cells to produce CBT-ol, leveraging DXP pathway overexpression [60]. After 5 days of

fermentation in a 5-L fermenter, they achieved a final concentration of 120 mg/L CBT-ol. Furthermore, Wang et al. introduced the IUP pathway into *E. coli*, systematically knocked out multiple endogenous pyrophosphatase genes, and enhanced membrane storage capacity through cell membrane engineering. Ultimately, they achieved a CBT-ol yield of 2.87 g/L in a 5 L bioreactor, which is the highest reported to date [61]. To improve P450 solubility in *E. coli*, the researchers fused *Taxus cuspidata* t-P450 with tobacco *n*-CPR; however, only trace amounts of CBT-diol were detected in shake flask cultures.

Ambrein

Ambrein, a triterpenoid primarily found in ambergris, is highly valued in the fragrance industry for its distinctive aroma. Owing to the scarcity of natural ambrein, research has focused on biosynthesis to meet demand while minimizing environmental impact. Ambrein biosynthesis relies on squalene as a precursor, with current efforts centered on engineering micro-organisms to synthesize squalene for conversion into ambrein. Ke et al. developed a synthetic pathway for the production of squalene-derived ambrein in *E. coli* [62]. They integrated *ScERG9* from *S. cerevisiae*, encoding squalene synthase (SQS), into the *E. coli* genome to enhance squalene supply. They then co-expressed a mutant squalene-hopene cyclase (SHC D377C), tetraisoprenyl- β -curcumene cyclase (*BmeTC*), and SQS in *E. coli* LKsb, achieving an ambrein yield of 2.6 mg/L.

Engineering yeasts for terpenoid flavor compound and precursor production

As eukaryote micro-organisms, yeasts possess specialized organelles, such as the endoplasmic reticulum, Golgi apparatus, and mitochondria, which enable them to perform intricate post-translational modifications [63]. This characteristic provides yeasts with a major advantage in synthesizing complex terpenoids, including β -carotene and lycopene [64, 65]. One of the key strategies in yeast-based terpenoid production is organelle compartmentalization, which involves anchoring key enzymes to specific organelles. This localized enzyme enrichment increases the concentration of intermediates, thereby enhancing synthesis efficiency. Furthermore, yeast metabolic networks exhibit greater stability, allowing sustained growth even in environments with high substrate and product concentrations, supporting continuous terpenoid production. In addition, yeasts offer several advantages for terpenoid biosynthesis: an ability to accommodate more complex enzymes, ease of achieving stable expression of multiple genes, and high chromosome integration efficiency [66]. Considering their structural complexity, metabolic flexibility, and robust industrial potential,

yeasts serve as superior microbial platforms for terpenoid biosynthesis. Moreover, they provide essential technical support for the sustainable and highly efficient synthesis of terpenoids. Table 2 summarizes the advancement in yeast-based synthesis of terpenoid flavor components and precursors recently, along with their aromatic properties.

Terpenoid flavor compounds

Linalool

Insufficient precursor supply and low catalytic efficiency of LIS are major limitations in the efficient synthesis of linalool. Zhou et al. addressed these challenges using protein and metabolic engineering. First, they overexpressed the MVA pathway and an engineered GPPS mutant to enhance precursor availability [67]. They then screened LIS variants and identified a high-activity mutant, t67OMcLIS, which was further optimized through directed evolution. Using *S. cerevisiae* as the host strain, they achieved a yield of 53.14 mg/L linalool in shake flasks. Further optimization introduced a highly active LIS mutant, t67OMcLis^{E343D/E352H}, and the ERG20^{F96W-N127W} variant was assembled. In addition, endogenous expression of Erg20 was downregulated, which further increased linalool production in shake flasks to 80.9 mg/L [68]. Zhang et al. introduced IUP in *S. cerevisiae* to enhance IPP and DMAPP production [69]. They genomically integrated the *S. cerevisiae* genes *IDII* and *ERG20*^{F96W-N127W}, and linalool synthesis was achieved using LIS (t26AaLS1) from *Actinidia arguta*. After optimizing substrate concentrations and using a bidirectional culture strategy, the final linalool yield was 142.88 mg/L after 72 h of shake flask fermentation.

α -Terpineol

α -Terpineol, a monoterpenoid alcohol with floral and citrus-like aromas, is synthesized through α -terpineol synthase (TPS). Improving TPS expression and catalytic efficiency is key to increasing production yields. Zhang et al. engineered an *S. cerevisiae* cell factory for α -terpineol production. By optimizing overexpression of the *tHMG1*, *IDII*, and *ERG20*^{F96W-N127W} genes, they increased α -terpineol titers to 0.83 mg/L [70]. To further enhance production, they fused *ERG20*^{F96W-N127W} with a truncated α -terpineol synthase from *Vitis vinifera* using a GSGSGSGSGS linker, resulting in strain LCB07. Compared with the parental strain LCB03, the functional expression of the fusion protein in LCB07 increased α -terpineol yield by 2.87-fold, reaching 2.39 mg/L. However, attempts to boost FPP accumulation through *ERG9* downregulation and *LPP1/DPP1* deletion did not improve yields. Instead, *ERG9* overexpression further elevated α -terpineol titers to 3.32 mg/L, forming strain

Table 2 Synthesis levels and aroma profiles of terpenoid flavor compounds and fragrance precursors in yeast

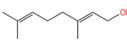
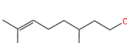
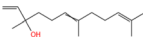
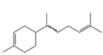
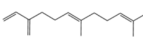
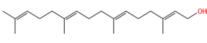
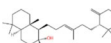
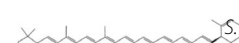
Product	Chemical formula	Host	Titers (g·L ⁻¹)	Scale	Productivity (g·L ⁻¹ ·h ⁻¹)	Aroma description	References
α-Terpineol		<i>S. cerevisiae</i>	21.88 mg·L ⁻¹	5-L fed batch	0.18 mg·L ⁻¹ ·h ⁻¹	Lilac fragrance; delicate and fleeting	[70]
Geraniol		<i>S. cerevisiae</i>	9.50	shake flask	0.079	Resembling the scent of roses; a lingering fragrance with a subtle bitterness	[134]
Borneol		<i>S. cerevisiae</i>	0.75	shake flask	4.50 mg·L ⁻¹ ·h ⁻¹	Intense fresh camphor aroma, with subtle woody and herbal undertones	[156]
Citronellol		<i>S. cerevisiae</i>	8.30	5-L fed batch	0.072	Fresh rose-like aroma with bitter notes	[157]
β-Ionone		<i>Y. lipolytica</i>	0.98	3-L fed batch	2.40 mg·L ⁻¹ ·h ⁻¹	Sweet floral and woody aroma with creamy and fruity notes	[112]
Nerolidol		<i>S. cerevisiae</i>	7.01	5-L fed batch	0.049	Subtle floral fragrance, reminiscent of roses and apples, characterized by sweetness, freshness, and longevity	[79]
(+)-Nootkatone		<i>S. cerevisiae</i>	2.39	3-L fed batch	0.016	Rich aroma of grapefruit peel, characterized by a fresh and vibrant scent, complemented by subtle bitter citrus notes, with warm woody and slightly earthy undertones	[93]
Valencene		<i>S. cerevisiae</i>	5.61	3-L fed batch	0.032	Rich citrus aroma with a woody undertone	[136]
(-)-Germacrene D		<i>S. cerevisiae</i>	7.90	5-L fed batch	0.072	Typical woody and spicy notes dominate, its odor is strong and well-diffused	[158]
Patchoulol		<i>S. cerevisiae</i>	1.63	5-L fed batch	0.018	Intense resinous aroma and herbal fragrance with minty, woody base notes	[159]
α-Bisabolene		<i>S. cerevisiae</i>	18.60	5-L fed batch	0.12	Warm woody, citrusy, floral, fruity, and green notes with a sweet balsamic aroma	[160]
Santalol		<i>S. cerevisiae</i>	1.30	5-L fed batch	0.018	Creamy and woody aroma	[76]
β-Farnesene		<i>S. cerevisiae</i>	130.00	20-m ³ fed batch	2.24	Green and subtly sweet, featuring a pronounced honey-wax floral aroma with a distinct citrus undertone	[10]
β-Caryophyllene		<i>S. cerevisiae</i>	21.40	3-L fed batch	–	Distinctive spicy, woody, and clove-like aroma	[88]
Geranylgeraniol		<i>S. cerevisiae</i>	6.33	5-L fed batch	0.044	Woody and herbal aromas, with mild floral and citrus notes	[91]

Table 2 (continued)

Product	Chemical formula	Host	Titers (g·L ⁻¹)	Scale	Productivity (g·L ⁻¹ ·h ⁻¹)	Aroma description	References
Sclareol		<i>Y. lipolytica</i>	12.90	5-L fed batch	0.054	Ambergris-like scent; a subtle yet powerful aroma, with a lasting presence; endowing a vivid, harmonious, and enduring fragrance	[94]
Ambrein		<i>P. pastoris</i>	0.11	5-L fed batch	1.40 mg·L ⁻¹ ·h ⁻¹	Oxidation and degradation yield aromatic compounds, such as ambroxide, with a floral and vegetable oil-like fragrance	[96]
β-Carotene		<i>Y. lipolytica</i>	39.50	3-L fed batch	0.17	Degradation results in a distinct caramelized aroma, with a subtle earthy scent, possibly accompanied by a faint baking or smoky odor	[103]
Lutein		<i>S. cerevisiae</i>	595.30 μg·L ⁻¹	shake flask	6.20 μg·L ⁻¹ ·h ⁻¹	Subtle grassy and vegetal aroma, complemented by a faint herbal fragrance and slight bitterness following degradation	[104]
Cembratriene-diol		<i>S. cerevisiae</i>	1.05 mg·L ⁻¹	3-L fed batch	0.015 mg·L ⁻¹ ·h ⁻¹	Roasted aroma, with a slightly sweet herbal scent and cocoa fragrance	[161]

LCB08. Using batch and fed-batch fermentation in a 5-L bioreactor, they achieved a final α-terpineol yield of 21.88 mg/L.

Geraniol

Geraniol synthesis in yeast is less efficient than that in *E. coli*, likely due to complex metabolic networks in yeast and competing pathways, e.g., the diversion of IPP and DMAPP into the ergosterol synthesis pathway. Although modulating ergosterol metabolism has improved production, competition from branch pathways continues to limit geraniol yields [71]. Zhao et al. used *Candida glycerinogenes* as a host and implemented dual-pathway engineering, achieving geraniol production of 858.4 mg/L when supplemented with 40 mM isopentenol [72]. To further reduce production costs, they developed a UPC2-mediated ergosterol feedback regulation system, which autonomously redirected carbon flux toward geraniol synthesis. By modifying the ergosterol promoter and optimizing transcription factor expression, they increased geraniol titers to 531.7 mg/L. To further enhance production, they engineered a xylose assimilation pathway in *C. glycerinogenes* and improved the pentose phosphate pathway, supporting efficient xylose

metabolism. Using glucose as the carbon source, geraniol titers reached 1091.6 mg/L in a 5-L fermenter [73].

α-Santalene

α-Santalene, an essential sesquiterpene widely used in fragrances, has been subject to major improvements in yeast-based metabolic engineering [74]. Scalcinati replaced the native promoter of SQS (ERG9) with HXT1, which efficiently regulated ERG9 expression and redirected carbon flux toward α-santalene synthesis [75]. They also knocked out the *LPP1* and *DPP1* genes, which are involved in competing metabolic pathways, and overexpressed ERG20, achieving an α-santalene yield of 92 mg/L, representing a 3.4-fold improvement over the initial strain. Zha et al. also substituted the ERG9 promoter with HXT1, but instead of deleting competing pathways, they overexpressed GAL4, a transcriptional activator of the GAL promoter, to enhance α-santalene synthesis [76]. By increasing the expression of yeast phosphoglucomutase, they further improved galactose uptake, leading to increased α-santalene production. Ultimately, by introducing the α-santalene synthase *SaSS* from *Santalum album*, they achieved a maximum yield of 1.3 g/L. Zhang et al. screened FPPS variants to improve precursor flux and performed site-directed mutagenesis on

α -santalene synthase, enhancing soluble enzyme expression and optimizing downstream pathways [77]. Furthermore, a fusion tag was introduced to the mutant enzyme, achieving final α -santalene titers of 1292 mg/L in shake flasks and 2916 mg/L in fermenters.

Nerolidol

Multifaceted metabolic reprogramming strategies have been developed to overcome the inherent metabolic constraints of *S. cerevisiae*, in which endogenous FPP is predominantly channeled toward ergosterol biosynthesis, and address the limitations imposed by its complex regulatory network on nerolidol production. Qu et al. enhanced the metabolic flux of *S. cerevisiae*'s MVA pathway and implemented a dual-induction system using the GAL promoter to synthesize nerolidol [78]. Overexpressing the transcription factor HAC1 increased nerolidol yield to 497.0 mg/L in shake flasks. Li et al. constructed an *S. cerevisiae* cell factory for high nerolidol production [79]. They overexpressed the MVA pathway through the GAL promoter, knocked out the *GAL80* gene to optimize the glucose response of the GAL regulatory systems, and integrated *UPC2-1* at the *GAL80* locus. In addition, they replaced the native *ERG9* promoter with *HXT1* (a glucose-sensitive promoter) to reduce FPP consumption, achieving a final yield of 7.01 g/L in a fermenter.

β -Caryophyllene

β -Caryophyllene is a bicyclic sesquiterpene that has a mild clove-like aroma. It is widely used in fragrances, flavors, pharmaceuticals, and cosmetics and is also considered a promising biofuel because of its high energy density [80–82]. Previous studies have demonstrated the microbial synthesis of β -caryophyllene; however, the yields have not yet reached the levels necessary for commercial viability [83–86]. Li et al. identified and cloned four β -caryophyllene synthase genes from the traditional Chinese medicinal plant *Artemisia argyi*, with *AarTPS88* showing the highest catalytic activity [87]. Using *S. cerevisiae* as a host, they co-expressed *ERG20* with *AarTPS88* to enhance the flux of the precursor FPP. Using a metabolic engineering strategy in a 5-L bioreactor through fed-batch fermentation, the yield of β -caryophyllene reached 15.6 g/L. Zhang et al. adopted a system engineering approach to regulate the sterol biosynthesis pathway of *S. cerevisiae*, thereby significantly enhancing β -caryophyllene production [88]. The key gene promoters (*ERG9*, *ERG1*, *ERG7*, and *ERG11*) were replaced with the glucose-responsive promoter *P_{HXT1}* to redirect the carbon flux toward β -caryophyllene synthesis. Furthermore, the promoters of fatty acid synthesis genes (*ACC1*, *FAS1*, and *FAS2*) were substituted with *P_{ERG7}* to minimize metabolic competition. Furthermore, the

glucose-sensing pathway was fine-tuned by modulating the expression of the transcription factor *Rgt1* and the protein kinase *Tpk2*. Finally, diploid fusion was applied to restore growth performance. The final engineered strain achieved a β -caryophyllene titer of 21.4 g/L in fed-batch fermentation. This study describes an efficient cell factory for the industrial production of β -caryophyllene and offers a strategy for the synthetic biological modification of other terpenoid compounds.

Geranylgeraniol

Geranylgeraniol (GGOH), a diterpenoid with mild floral and citrus aromas, is primarily derived from *Bixa orellana*. Advancements in GGOH synthesis have focused on constructing cell factories using yeast strains, including *S. cerevisiae* and *Y. lipolytica*. These efforts enhanced carbon flux toward GGOH by downregulating squalene biosynthesis and increasing the supply of precursors (e.g., IPP), among other strategies [89]. Wang et al. used *Y. lipolytica* as a host, enhancing GGOH synthesis by increasing FPP, GGPP, and acetyl-CoA availability while downregulating squalene biosynthesis through promoter engineering [90]. By optimizing the fermentation conditions and reducing reactive oxygen species production, they achieved a GGOH titer of 3346.67 mg/L in shake flasks. Further improvements introduced NADH-dependent HMG-CoA reductase from *Silicibacter pomeroyi* to enhance NADH utilization, along with the overexpression of *PaGGPPs-ERG20*, *PaGGPPs-DPP1*, and the MVA pathway as well as the downregulation of *ERG9* expression. Using *S. cerevisiae*, they achieved a yield of 6.33 g/L GGOH in a bioreactor [91].

Nootkatone

Yeast is more suitable than *E. coli* for nootkatone synthesis owing to its superior P450 enzyme functionality. Its endoplasmic reticulum supports proper enzyme folding and cofactor availability, and native P450 expression enhances heterologous P450 stability and activity [92]. Liu et al. engineered *S. cerevisiae* using a high-throughput screening system based on 2,4-dinitrophenylhydrazine-carbonyl reactions to identify candidate P450 oxygenase (HPO) mutants [93]. Semi-rational engineering of HPO and P450 reductase increased catalytic efficiency by 2.54-fold with the optimal mutant HPO_M18. They further knocked out *ERG9* and the transcription factor *ROX1* to inhibit squalene synthesis and overexpressed key enzymes in the MVA pathway, namely, tHMG1 and *ERG12*. In addition, *CnVS*, *HPO_M18*, and *ADHm* were introduced. This resulted in a (+)-nootkatone yield of 2.39 g/L through two-stage, fed-batch fermentation, the highest yield reported to date.

Sclareol

Strategic integration of enzyme engineering for catalytic optimization with metabolic network rewiring to augment precursor flux constitutes the predominant paradigm for high-titer sclareol biosynthesis in yeast-based platforms. Sun et al. engineered a biosynthetic sclareol production pathway in *Y. lipolytica* by evaluating multiple truncated and full-length sclareol synthases (Lpps), with the truncated *tSsLpps* exhibiting the highest catalytic efficiency [94]. To further enhance precursor availability and minimize substrate diffusion losses, they engineered a novel pathway and scaffold-free enzyme complex. This approach led to a final sclareol yield of 12.9 g/L after 240 h of fermentation. Cao et al. initially fused the diterpene synthases Lpps and TPS and incorporated maltose-binding protein to enhance the stability of the fusion protein. Next, they reconfigured the metabolic network to increase the availability of acetyl-CoA and GGPP while suppressing competing metabolic pathways. High-density fermentation in a 5-L bioreactor resulted in a final sclareol yield of 11.4 g/L. This study demonstrated the efficient microbial biosynthesis of sclareol and validated the feasibility of the theoretical framework known as “metabolic module adaptability.” These results provide a foundation for the rational design and engineering of high-performance microbial cell factories [95].

Flavor precursors

Ambrein

Yeast-based synthesis is well-suited for ambrein production. Moser et al. enhanced squalene accumulation in *Pichia pastoris* by downregulating squalene epoxidase (ERG1) and reducing metabolic flux toward ergosterol [96]. They engineered *BmeTC* D373C, a mutant with superior catalytic efficiency compared to the cascade reaction involving *BmeTC* D373C and *BmeTC*. Using strain P_{PISI}-*ERG1* TC D373C, fermentation in a 5-L bioreactor over 74 h yielded >100 mg/L ambrein. Lin et al. developed an engineered yeast strain for de novo ambrein production by optimizing the MVA pathway [97], overexpressing codon-optimized *BmeTC* and its mutant *BmeTC*^{Y167A/D373C}, testing various promoters, and knocking out *GAL80*. However, the low catalytic efficiency of *BmeTC* remained a bottleneck. To overcome this, they modified the surface amino acids of *BmeTC*, generating the variant *BmeTC*^{K6A/Q9E/N454A}. This resulted in a 457.4 mg/L ambrein yield, the highest reported in yeast to date.

β -Carotene

β -Carotene, a tetraterpenoid aromatic compound, is extensively used in various industries, including animal feed, food products, pharmaceuticals, and cosmetics

[98–101]. Lin et al. developed a high-yield β -carotene-producing *S. cerevisiae* strain by enhancing the MVA pathway genes, modulating squalene synthesis, and deleting *FLD1* to expand lipid droplet capacity, which resulted in a yield of 2.09 g/L β -carotene in a 5-L bioreactor [102]. MA et al. introduced two sources of phytoene desaturase and bifunctional phytoene synthase/lycopene cyclase [103]. They found that lycopene cyclase was inhibited by lycopene, creating a bottleneck in carotenoid biosynthesis. To overcome this, they developed two strategies that nearly eliminated lycopene-induced substrate inhibition and enhanced carotenoid production: (1) using phylogeny-based and structure-guided protein engineering, they generated mutants that eliminated substrate inhibition while maintaining enzyme activity, increasing β -carotene yield; and (2) establishing a GGPPS-mediated metabolic flux regulator to control lycopene formation, keeping intracellular lycopene levels below the “inhibition threshold” to prevent substrate inhibition and boost β -carotene yield. Ultimately, the engineered strain produced 39.5 g/L β -carotene in a fermenter.

Lutein

Lutein, a tetraterpenoid, has degradation products, such as β -ionone, that contribute to floral and fruity aromas, making it valuable in the fragrance industry. In lutein biosynthesis, lycopene undergoes asymmetric cyclization through lycopene cyclase to form α -carotene, which is hydroxylated by carotenoid hydroxylase to produce lutein. However, the preference of lycopene cyclase for symmetric cyclization, leading to β -carotene formation, creates a major bottleneck. To mitigate metabolic flux loss from competition in the β -cyclization branch, Bian et al. implemented a sequential expression strategy using two lycopene cyclases, regulated by the temperature-sensitive Gal4M9 protein [104]. This approach redirected metabolic flux toward α -carotene production. In addition, ϵ -cyclase was engineered and relocated to the plasma membrane to further optimize α -carotene synthesis. The pathway was enhanced by incorporating carotene hydroxylase CYP97A3 and Lut1 from *Arabidopsis thaliana*, with α -carotene hydroxylation efficiency improved by adjusting CYP97A3 copy numbers. These optimizations led to a lutein yield of 595.3 μ g/L in yeast.

Strategies for enhancing terpenoid biosynthesis

Microbial cell factories offer a promising approach to terpenoid production given their short cycle, ease of operation, and low cost [105, 106]. This method also supports sustainability, high efficiency, enantiomeric purity, and scalability. Despite major progress in microbial fermentation, increasing terpenoid yield remains challenging owing to uncoordinated gene expression in metabolic

pathways [107], low enzyme activity [108], and limited precursor availability [109]. With the rapid advancements in synthetic biology, a range of effective biosynthetic strategies have been developed to address these challenges. These approaches enhance precursor supply and optimize metabolic flux toward terpene biosynthesis [2], laying a solid foundation for improving the yield of terpenoid flavor compounds and aroma precursors.

Modular pathway engineering

Engineering micro-organisms for terpenoid synthesis requires regulating endogenous pathways and, in some cases, introducing exogenous pathways. These modifications can disrupt the metabolic flux, affecting target compound yields. Addressing these metabolic flux perturbations remains a key challenge [110]. This modular design breaks down complex pathways into distinct

functional units, such as modules for upstream precursor supply, core scaffold synthesis, and downstream product modification, allowing hierarchical regulation and flexible adjustment of metabolic fluxes, which significantly improves the efficiency of terpene synthesis. Furthermore, modular design offers a rational solution by maintaining high carbon flux during synthesis of the C5 intermediate, isopentenyl pyrophosphate. By modifying downstream modules of the intermediate, various terpenoids can be efficiently produced, improving overall yield (Fig. 2A). For example, Zhang et al. developed a four-module apocarotenoid synthesis platform in *E. coli*, which consisted of the upstream MVA pathway (module 1), downstream MVA pathway (module 2), lycopene biosynthesis module (module 3), and apocarotenoid modification module (module 4) [111]. Using experimental design-assisted system

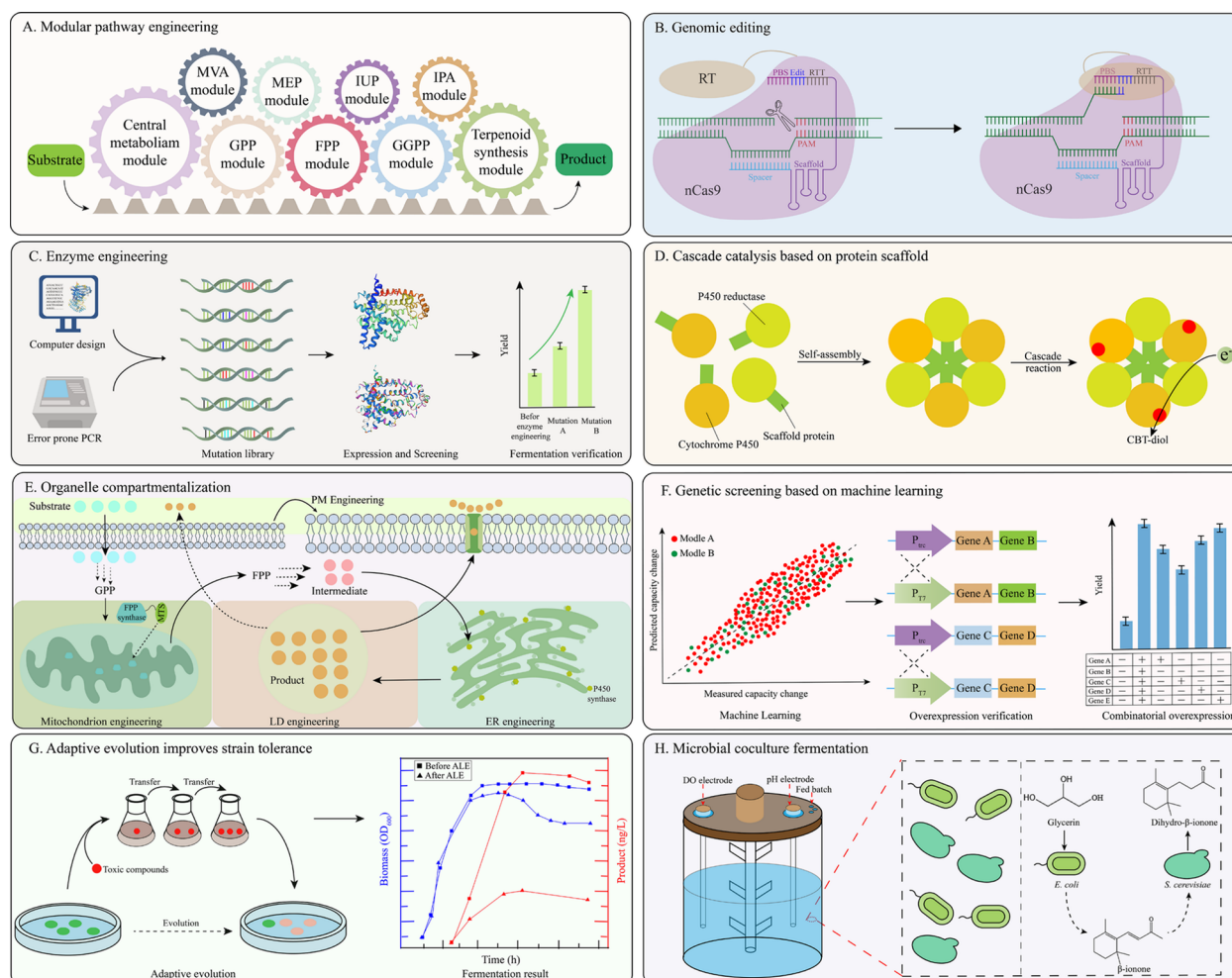


Fig. 2 Strategies to enhance the biosynthetic yield of terpenoids. **A** Modular pathway engineering; **B** genomic editing (CRISPR/Cas9/Cpf1); **C** enzyme engineering; **D** cascade catalysis based on protein scaffold; **E** organelle compartmentalization, MTS: mitochondrial targeting signaling peptide; **F** genetic screening based on machine learning; **G** adaptive evolution improves strain tolerance evolution; and **H** microbial coculture fermentation.

pathway optimization, the metabolic flux among the modules was effectively balanced, resulting in a lycopene yield of 107 mg/L under shake flask conditions. By substituting different combinations of enzymes in module 4 (e.g., LCYe-CCD1 → crtY-CCD1 → crtY-BCDO), a “plug-and-play” strategy enabled the production of α -ionone (480 mg/L), β -ionone (500 mg/L), and retinol (33 mg/L). Lu et al. applied modular pathway engineering to enhance β -ionone synthesis in *Y. lipolytica* [112], dividing the biosynthetic pathway into three modules: the precursor acetyl-CoA synthesis module, the MVA pathway module, and the β -ionone synthesis pathway module. Through pathway design and assembly, they constructed a high-yield strain. Fed-batch fermentation in a 3-L fermenter produced a maximum β -ionone yield of 0.98 g/L. In conclusion, modular design enables the systematic coordination of multiple enzyme expressions and offers a versatile framework for enhancing the diversity of downstream products.

Genomic editing

CRISPR/Cas9, derived from the Type II CRISPR/Cas system, simplifies gene editing in micro-organisms, including those that are traditionally difficult to modify. It enables precise insertions, deletions, and mutations, facilitating metabolic pathway engineering in prokaryotic and eukaryotic systems. Thus, CRISPR/Cas9, in addition to variants, such as CRISPRi and CRISPRa, promotes increased biosynthesis of terpene flavor compounds (Fig. 2B).

Using CRISPR/Cas9 for microbial genome editing, metabolic networks can be modified through gene knockouts or insertions to redirect carbon flux toward target metabolites and enhance product yield. Eaucalire et al. used CRISPR/Cas9 to integrate 17 fragments into the *S. cerevisiae* genome, successfully constructing a complete β -carotene biosynthetic pathway [113]. In addition, CRISPR/Cas9-based approaches enable precise regulation of target genes to boost terpenoid synthesis. Lian et al. used CRISPRa to upregulate *HMG1*, a gene encoding a rate-limiting enzyme in the mevalonate pathway, and used CRISPRi to downregulate *ERG9* in the β -carotene and sterol biosynthesis branch pathways [114]. They also knocked out the transcriptional regulatory factor gene *ROX1*, achieving a threefold increase in β -carotene synthesis. Zhang et al. further improved yields by overexpressing MVA pathway genes and introducing multiple copies of β -carotene biosynthesis genes via CRISPR/Cas9 [115]. Combined with optimized fermentation conditions, this approach resulted in a β -carotene yield of 4.5 g/L.

Enzyme engineering

In terpenoid biosynthesis, the low catalytic activity, poor specificity, and insufficient stability of key enzymes are major factors limiting yield. Strategies such as directed evolution, rational design, and semi-rational design are commonly used to construct mutant libraries and identify optimal enzymes. However, these methods require an in-depth understanding of protein structure and function and are often labor-intensive [116]. To overcome these limitations, efforts in recent years have focused on protein fusion and chemical modification as effective strategies for enhancing enzyme activity and increasing target product yields [117] (Fig. 2C, and D). Park et al. developed an innovative electron conduction strategy using the *Photorhabdus luminescens* crystal protein CipB as a scaffold [118]. By assembling multiple enzymes into functional protein crystalline inclusions, this approach facilitated direct enzyme–enzyme interactions and enabled efficient electron transfer between P450s and reductases. Applying this strategy in *E. coli* for lutein biosynthesis, they optimized the heme pathway and fermentation conditions, achieving a lutein yield of 218 mg/L within 2 days. Similarly, Kang et al. improved the efficiency of cascade reactions in micro-organisms using the spherical protein cage Mi3 [119]. Using *E. coli* as a host, they biosynthesized lycopene and used the protein cage system to anchor multiple pathway enzymes to the Mi3 surface. This strategy resulted in an 8.5-fold increase in lycopene production. Increasing product yields and ensuring cost-effective production are essential for widespread application.

Organelle compartmentalization and transporter engineering

In eukaryotic micro-organisms, such as *S. cerevisiae*, organelle compartmentalization has been used to enhance terpenoid synthesis by taking advantage of the distinct physicochemical conditions of specific organelles (Fig. 2E) [120, 121]. By anchoring key enzymes to organelles, such as the endoplasmic reticulum, mitochondria, and plasma membrane, local enzyme and intermediate concentrations are increased, improving synthesis efficiency. Several engineering strategies have been successfully implemented, such as endoplasmic reticulum engineering [122], lipid droplet engineering [123, 124], peroxisome engineering [125, 126], mitochondrial engineering [127], and plasma membrane engineering [128]. A major limitation in microbial terpenoid biosynthesis is the cytotoxicity of accumulated target products, which reduces cell viability and hinders efficient production [129]. Transporter engineering has emerged as a viable solution, involving the upregulation of ABC transporter

expression and the development of artificial membrane vesicle trafficking systems (AMVTS) to facilitate the secretion of terpenoid compounds into the extracellular space [130, 131]. These approaches could also facilitate the recovery of the target products.

Matsumoto et al. hypothesized that the high concentration of acetyl-CoA in mitochondria could enhance carotenoid biosynthesis [132]. By relocating the carotenoid biosynthetic pathway to mitochondria, they achieved a 13.82-fold increase in carotenoid yield compared with that of cytoplasmic engineering strains. Dusseaux et al. found that the introduction of a heterologous MVA pathway, along with GPPS and monoterpene synthases, into peroxisomes enhanced the conversion of acetyl-CoA into valuable monoterpenoids. This strategy is more efficient than cytoplasmic production. If DMAPP, IPP, and GPP are not rapidly used within the peroxisome, they can be transported back to the cytoplasm through transporters, such as Pxmp2. This mechanism reduces intermediate loss and sustains a high local substrate concentration, thereby increasing the production yield [133]. Baker et al. reconstructed the MVA pathway in *S. cerevisiae* peroxisomes by implementing a dual-compartment fermentation strategy to coexpress it with the cytoplasmic pathway for geraniol production [134]. Peroxisomal capacity was directly correlated with synthesis efficiency. By overexpressing key genes and integrating machine learning approaches (Fig. 2F), they enhanced peroxisomal capacity by 1.37-fold, ultimately achieving a geraniol yield of 9.5 g/L under fed-batch conditions. Excessive intracellular terpenoid accumulation can impair cellular activity, thereby reducing biosynthetic efficiency, particularly for cytotoxic terpenoids. To address this, transport protein engineering strategies facilitate the secretion and recovery of target products while minimizing their adverse effects. Wu et al. developed the AMVTS in *E. coli*, using membrane lipids for the transport of hydrophobic molecules, effectively increasing hydrophobic terpenoid secretion [131]. Their study also identified lipid proteins, such as TolR and NlpI, as key regulators of outer membrane vesicle formation. Combining the AMVTS method with *tolR* and *nlpI* knockouts led to a 13.7% increase in β -carotene secretion.

Adaptive laboratory evolution

Adaptive laboratory evolution (ALE) is an undirected genomic modification strategy (Fig. 2G). Considering that certain terpenoids exhibit cytotoxicity, which limits synthesis efficiency, ALE can enhance the tolerance of chassis cells to high concentrations of these compounds. Niu et al. enhanced the tolerance and yield of *E. coli* to α -pinene using the ALE strategy. They subjected *E. coli* to atmospheric and room-temperature plasma mutagenesis

treatment and gradually increased the concentration of α -pinene in the culture medium from 0.5% to 2.0% for adaptive selection. They obtained a strain that was tolerant of 2.0% α -pinene and increased its yield by 31% [135]. Zhu et al. used *S. cerevisiae* with mannitol as the carbon source to engineer a cell factory for efficiently producing valencene [136]. Wild-type *S. cerevisiae* cannot utilize mannitol; therefore, the researchers applied adaptive evolution to a previously engineered valencene-producing strain, yielding the mutant strain BN-91A, which could grow on mannitol-based media. Compared with glucose, mannitol tripled valencene production. Further optimization of precursor supply, substrate uptake, and cofactor regeneration led to a final yield of 161.1 mg/L in shake flasks and 5.61 g/L in a 3-L fermenter. Limonene's cytotoxicity also poses a challenge for efficient biosynthesis. Li et al. used transcriptomics to identify eight genes in *Y. lipolytica* that improved tolerance to limonene [137]. Overexpression of YALI0F19492p increased limonene production eightfold, demonstrating that tolerance and evolutionary engineering can markedly enhance limonene yield and host strain robustness.

Bioderivatization

The enzymatic derivatization strategy effectively alleviates product toxicity and significantly improves synthesis efficiency [138, 139]. This approach involves the expression of specific transferases, such as alcohol acetyltransferases or glycosyltransferases, which catalyze the conversion of hydrophobic terpenoids, particularly toxic compounds, such as geraniol, into low-toxicity derivatives, such as acetates or glycosides. The target end-products can then be reverted to their original chemical forms through hydrolysis, which is an important step in the product recovery process [28]. For example, Priebe et al. developed a byproduct-free, whole-cell catalytic process for geraniol glycosylation and demonstrated that enzymatic detoxification can enhance the biosynthesis of terpenoids [140]. Wang et al. successfully converted cytotoxic geraniol into the less harmful geranyl acetate by expressing alcohol acetyltransferase from *Rosa hybrida* (RhAAT). This enabled host cells to sustain normal growth at geraniol-equivalent concentrations up to 700 mg/L [141]. Through systematic optimization of fusion protein design, amplification of AAT gene copy number, and glycerol concentration-controlled fed-batch fermentation, they achieved the highly selective production of geranyl acetate at 10.36 g/L in a 1.3-L bioreactor. This integrated strategy not only significantly reduced geraniol-induced toxicity but also improved carbon conversion efficiency to 0.126 g/g glycerol. Similarly, Shukal et al. reported *in situ* acetylation of geraniol to geranyl acetate by coexpressing *RhNUDX1* and *RhAAT1* [30].

A final geranyl acetate titer of 19.0 g/L was obtained from a 5-L bioreactor after systematic fermentation optimization.

Coculture systems

Owing to the complexity of terpene biosynthetic pathways, a single chassis cell often fails to provide optimal expression of all key enzymes. Effective expression of these enzymes is crucial for maintaining flux balance, minimizing metabolic burden, and enhancing catalytic efficiency. Introducing a coculture system divides the biosynthetic pathway into independent catalytic modules, with each module hosted by an appropriate cell type [142]. This approach addresses challenges related to complexity (Fig. 2H), offering several advantages: (1) minimizing interference between pathway modules; (2) reducing the metabolic load on each host cell; (3) allowing precise optimization of each module by adjusting strain ratios; and (4) enabling flexible, plug-and-play biosynthesis of diverse products. Qi et al. used *E. coli* to synthesize β -ionone but observed excessive accumulation of dihydro- β -ionone without complete conversion [143]. To resolve this, they introduced a coculture system, where *E. coli* produced β -ionone and *S. cerevisiae* expressing the DBR1 module converted it to dihydro- β -ionone. Optimizing strain ratios elevated the yield to 27 mg/L, representing a 3.4-fold increase compared with using a single strain. Tang et al. developed a modular coculture system for sclareolide production using *S. cerevisiae* and *Cryptococcus neoformans* ATCC 20918 [144]. First, a recombinant *S. cerevisiae* strain was engineered to produce 536.2 mg/L of sclareol. Subsequently, *Hyphozyma roseo-ignea* was used to catalyze its conversion to sclareolide. By optimizing coculture conditions, the final yield reached 626.3 mg/L, emphasizing the potential of coculture strategies for sustainable sclareolide biosynthesis.

Fermentation optimization strategies

In the industrialization of microbial synthesis for terpenoid fragrances, bioreactor fermentation is an important method for evaluating the physiological characteristics and economic productivity of microbial strains. However, during the scale-up process, variations in environmental parameters, such as increased shear stress and formation of dissolved oxygen gradients, can result in the so-called “scale effect,” which disrupts metabolic flux and reduces product synthesis efficiency [145, 146]. To mitigate these adverse effects, strain adaptation strategies have proven effective. For example, Huang et al. examined the synthesis of α -ionone using *E. coli* and found that a significant amount of H_2O_2 was generated during large-scale cultivation. This reactive species caused oxidative degradation of lycopene, a key precursor, thereby reducing the overall

yield. To overcome this problem, they overexpressed alkyl hydroperoxide reductase AhpC/F in the engineered strain to efficiently remove excess H_2O_2 , thereby restoring lycopene accumulation. This resulted in a yield of 700 mg/L of α -ionone in a 5-L bioreactor, demonstrating enhanced oxidative stress tolerance and improved stability for large-scale production [147].

The two-stage fermentation strategy has been widely adopted in fermentation. By separating the microbial growth phase from the product synthesis phase, this approach facilitates the efficient biosynthesis of terpenoid flavor compounds. The fundamental principle of this strategy involves isolating biomass accumulation, which predominantly occurs during the exponential growth phase under favorable nutritional and environmental conditions, from targeted product formation, which occurs during the stationary phase through interventions, such as carbon source switching, temperature or pH modulation, and activation of inducible promoters within the metabolic pathway. By alleviating the constraints imposed by growth-associated carbon flux allocation and redirecting metabolic flux toward the desired biosynthetic route, this method significantly enhances the spatiotemporal productivity and process robustness [148]. Zhang et al. used dynamic regulation of key genes in the sterol biosynthesis pathway using a glucose-responsive promoter to establish an effective two-stage fermentation system [88]. During the glucose-rich phase, competing pathways were suppressed to prioritize cell proliferation. Upon glucose depletion, these inhibitory effects were relieved automatically, thereby initiating β -caryophyllene biosynthesis. In fed-batch fermentation, an initial glucose-containing medium was used to enhance biomass generation, followed by a switch to an ethanol-based medium combined with in situ extraction techniques, which markedly improved the efficiency of metabolic flux redirection. The resulting yield of β -caryophyllene increased by 10.5-fold compared with that of the original strain, reaching 21.4 g/L. This demonstrates the substantial benefits of the two-stage fermentation strategy for terpenoid compound synthesis.

Optimizing the fermentation process through precise dynamic regulation of key environmental parameters, such as pH, dissolved oxygen, and temperature, as well as strategies such as two-phase cultivation can alleviate external stress and provide a more favorable growth environment for micro-organisms. This facilitates stable or even high-yield production of terpenoid compounds [149]. For example, Shukal et al. demonstrated that microaerobic fermentation can serve as an effective strategy to overcome yield limitations by modulating redox homeostasis and metabolic flux distribution. The underlying mechanism involves the significant inhibition

of geraniol oxidative degradation into citronellal under low-oxygen conditions, which also reduces the metabolic burden. By replacing traditional dissolved oxygen control with redox potential regulation in a 5-L fermenter and precisely maintaining a low-oxygen reducing state through the dynamic adjustment of aeration and agitation rates, the geranyl acetate yield was increased to 19 g/L [30]. Furthermore, because of the highly hydrophobic nature of most terpenoid flavor compounds, the application of two-phase fermentation for in situ extraction not only enhances product stability but also significantly boosts synthesis efficiency. In a study of trans-nerolidol synthesis using *E. coli* by Tan et al., the implementation of a dodecane-based two-phase extraction system markedly improved large-scale production performance. In particular, the organic phase efficiently captured 94% of the hydrophobic products from the aqueous phase while reducing gas-phase volatilization loss by over 3.4%. This enabled in situ product removal, alleviated intracellular accumulation inhibition, and increased the yield from 7 g/L in single-phase fermentation to 16 g/L within 93 h in a 5-L bioreactor, resulting in an improvement of 128% [47].

In conclusion, strategies for regulating the microbial environment and optimizing the fermentation process are important for overcoming the “scale effect.” By implementing approaches, such as microaerobic fermentation, two-phase extraction, and two-stage cultivation, the synthesis efficiency and process robustness of terpenoids may be significantly enhanced.

Outlook

Although significant progress has been made in the production of certain terpenoids, such as geraniol and limonene, through biosynthetic approaches, current production costs for these compounds remain higher than those incurred using conventional chemical synthesis methods. For many other high-value terpenoids (e.g., ambrein), biosynthesis yields and cost efficiency are below the thresholds required for commercial viability. Therefore, substantially enhancing biosynthetic efficiency to achieve economically competitive production levels is required for the future large-scale industrial adoption of microbial terpenoid biosynthesis. A major challenge is the low catalytic activity of terpene synthases, necessitating a deeper understanding of their native expression patterns and environmental conditions to optimize heterologous expression and fermentation processes. High-density fermentation is often required to achieve high terpenoid yields. However, many terpenoids exhibit cytotoxicity, and their excessive accumulation during fermentation can inhibit microbial cell growth. Consequently, strategies such as separating the growth and production

phases, enhancing host tolerance through adaptive evolution or genetic engineering, and leveraging unconventional hosts with higher solvent or pH tolerance could improve production. Nevertheless, gene editing tools for many unconventional hosts remain inefficient, highlighting the need for advanced genome engineering technologies. With the rapid advancements in synthetic biology and metabolic engineering, microbial cell factories have emerged as a highly promising platform for terpenoid biosynthesis, offering the potential to enable more scalable, sustainable, and cost-effective industrial production in the future.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author contributions

Q.L. writing—original draft, visualization, resources, investigation, data curation, and conceptualization; Y.N. writing—original draft, visualization, data curation; D.L., J.R., and C.L. resources and data curation; Z.C. writing—review and editing, supervision, project administration, and conceptualization; G.W. writing—review and editing, supervision, project administration, funding acquisition, and conceptualization. All authors reviewed the manuscript.

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Data availability

No data sets were generated or analyzed during the current study.

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