Reengineering *Zingiber zerumbet*: Recent Advances in Zerumbone Biosynthesis and Synthetic Biology

Irsalina Iwani Mohd Jazmi[1,2*], Hamid Baskar[1], and Nurul Huda Alwakil[3]

[1] Fasclone A.B.I Sdn Bhd, Bandar Tasik Puteri, Rawang, 48020, Selangor, Malaysia;
[2] Institute of Biological Sciences, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia;
[3] Plant Biotechnology Incubator Unit, University Malaya, Kuala Lumpur 50603, Malaysia;
*corresponding author: ybirsalinaiwani@gmail.com
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Abstract

Zingiber zerumbet is a medicinal plant known for producing zerumbone, a sesquiterpenoid with reported anticancer, anti-inflammatory, antimicrobial and antioxidant activities. Growing interest in zerumbone has increased the need for sustainable production methods, as conventional extraction from plant material is limited by low and inconsistent yields, long growth periods, and the need for large cultivation areas. Recent advances in synthetic biology have provided new opportunities for microbial production of zerumbone through metabolic engineering. This review summarizes current knowledge on zerumbone biosynthesis, focusing on the identification and functional characterization of the key enzymes ZSS1, CYP71BA1 and ZSD1. The successful reconstruction of the zerumbone biosynthetic pathway in engineered *Saccharomyces cerevisiae* is discussed, together with strategies used to enhance precursor supply and improve pathway efficiency. Lessons from the microbial production of other valuable sesquiterpenoids, including artemisinin, nootkatone, and patchoulol, are also examined to identify engineering approaches applicable to zerumbone. Finally, future perspectives involving protein engineering, CRISPR-based metabolic engineering, artificial intelligence-assisted enzyme design, and sustainable biomanufacturing are highlighted. Together, these approaches suggest that continued optimization of precursor supply, CYP71BA1 activity, and metabolic pathway balance will be essential for translating microbial zerumbone production into a commercially viable process.

Keywords: Zerumbone, Ginger, Synthetic Biology, Metabolic Engineering, Bioactive Compound

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Introduction

Zingiber zerumbet, commonly known as shampoo ginger, bitter ginger, or pinecone ginger, is a tropical perennial plant widely cultivated in tropical and subtropical regions due to its economic and medicinal value (Okamoto et al., 2011; Yu et al., 2011; Yu et al., 2008). Its rhizomes contain a variety of terpenoids, with zerumbone being the major sesquiterpenoid component of the essential oil. Unlike many other sesquiterpenoids that are mainly used as flavoring or fragrance compounds, zerumbone has attracted considerable attention because of its broad range of reported biological activities, particularly its anticancer and anti-inflammatory properties (Yang et al., 2026). This makes zerumbone a promising candidate for pharmaceutical development and an attractive target for synthetic biology research. Traditionally, the rhizomes have been used in folk medicine to treat ailments such as sprains, indigestion, toothaches, and inflammatory conditions (Zhang et al., 2018). In recent years, scientific interest in *Z. zerumbet* has increased because of the pharmacological properties of zerumbone. Studies have shown that zerumbone possesses anti-inflammatory and anticancer activities and can inhibit the growth of various cancer cell lines by inducing apoptosis. These effects have been observed in treating several types of cancer, highlighting the potential of zerumbone as a promising therapeutic compound (Yang et al., 2026).

The growing understanding of zerumbone's pharmacological benefits, coupled with increasing public interest in natural health products, is expected to drive a greater demand for this compound in the future. However, terpenoids are generally produced in relatively low quantities in plants. Conventional extraction from plant material often results in low yields and purity while requiring large amounts of biological resources (Meng et al., 2020; Ro et al., 2006; Zhang et al., 2018). In addition, the chemical synthesis of terpenoids is challenging and costly because of their complex molecular structures.

To overcome the limitations, advances in biotechnology have created new opportunities for the sustainable production of terpenoids through metabolic engineering (Meng et al., 2020; Ro et al., 2006; Yang et al., 2026; Zhang et al., 2018). The identification of the key biosynthetic genes, namely *ZSS1*, *CYP71BA1*, and *ZSD1*, has provided important insights into the enzymatic pathway responsible for zerumbone biosynthesis (Okamoto et al., 2011; Yu et al., 2011; Yu et al., 2008). This breakthrough has enabled researchers to reconstruct the complete biosynthetic pathway in engineered yeast strains, demonstrating the potential of microbial platforms for zerumbone production (Zhang et al., 2018). Besides, a study conducted by Alwakil et al. (2022) showed that a synergistic application of Methyl Jasmonate (MeJA) and Salicylic Acid (SA) in *Z. zerumbet* adventitious root culture produced a significant amount of zerumbone (43 mg/g) as compared to control (Alwakil et al., 2022). Although the current production levels remain relatively low due to challenges such as limited precursor supply and inefficiencies associated with cytochrome P450 enzymes, ongoing developments in metabolic engineering, pathway optimization, and synthetic biology offer promising avenues for enhancing production efficiency and achieving industrial-scale biosynthesis in the future.

Methodology

Biosynthesis of Zerumbone in Zingiber zerumbet

Zerumbone biosynthesis originates from the mevalonate (MVA) pathway, which generates the sesquiterpene precursor FPP. The terpene synthase ZSS1 converts FPP into α-humulene, which is subsequently oxidized by CYP71BA1 and further modified by ZSD1 to form zerumbone. The elucidation of these enzymes enabled reconstruction of the pathway in heterologous hosts (Zhang et al., 2018).

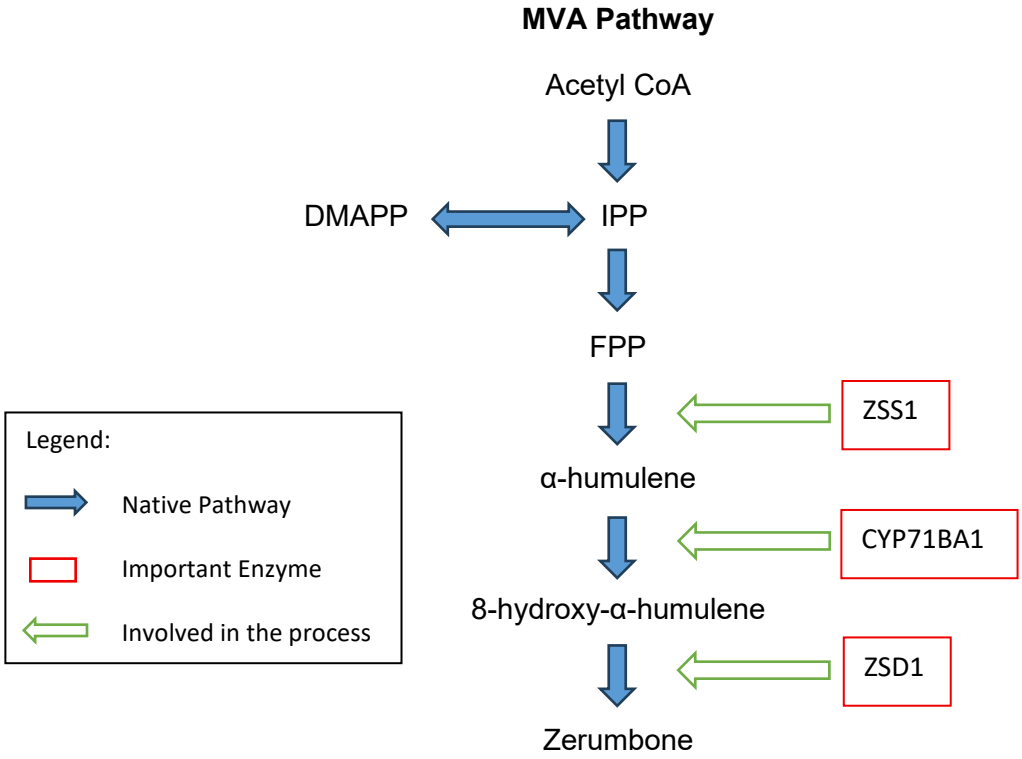

Figure 1: Schematic overview of Zerumbone synthesis in *Z. zerumbet*. IPP, isopentenyl pyrophosphate; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl diphosphate; ZSS1, α-humulene synthase; CYP71BA1, α-humulene 8-hydroxylase; ZSD1, zerumbone synthase (Zhang et al., 2018).

Terpenoid Precursor Formation

The biosynthesis of zerumbone begins with the production of the universal isoprenoid building blocks through the mevalonate (MVA) pathway. In this pathway, acetyl-CoA is converted through a series of enzymatic reactions into isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) (Figure 1). These five-carbon compounds serve as the fundamental precursors for terpenoid biosynthesis. Subsequent reactions produce farnesyl diphosphate (FPP), a 15-carbon intermediate that acts as the universal precursor for sesquiterpene biosynthesis. Since zerumbone is a sesquiterpenoid, its biosynthetic pathway originates from FPP.

Formation of α-Humulene

The first committed step in zerumbone biosynthesis is the cyclization of FPP into α-humulene (Figure 1). This reaction is catalysed by α-humulene synthase, encoded by the *ZSS1* gene (Yu et al., 2008). The identification of ZSS1 provided important evidence for the initial step of zerumbone biosynthesis and established α-humulene as the direct precursor of zerumbone.

Oxidative Modification by CYP71BA1

Following the formation of α-humulene, the molecule undergoes oxidative modification catalysed by the cytochrome P450 monooxygenase CYP71BA1. CYP71BA1 catalyses the hydroxylation of α-humulene to produce 8-hydroxy-α-humulene, representing a key oxidative step in zerumbone biosynthesis (Yu et al., 2011) (Figure 1). The discovery of CYP71BA1 was a significant milestone in elucidating the complete biosynthetic pathway of zerumbone.

Final Oxidation to Zerumbone

The final step in zerumbone biosynthesis is catalysed by zerumbone synthase dehydrogenase 1 (ZSD1). This enzyme oxidises 8-hydroxy-α-humulene to form zerumbone (Figure 1). During this process, the hydroxyl group is converted into an α,β-unsaturated carbonyl group, which is a characteristic structural feature of zerumbone and is believed to contribute significantly to its biological activities (Okamoto et al., 2011). The identification of ZSD1 completed the understanding of the key enzymatic steps involved in zerumbone biosynthesis and enabled the reconstruction of the pathway in engineered microbial hosts for potential large-scale production.

Discovery of Biosynthetic Genes and Enzymes

Terpene Synthases

The study of zerumbone biosynthesis started with the identification of terpene synthase (TPS) enzymes. TPS enzymes are responsible for forming terpene carbon skeletons from basic precursors such as farnesyl pyrophosphate (FPP). In *Zingiber zerumbet*, the main TPS involved in zerumbone production is α-humulene synthase (ZSS1). This enzyme converts FPP into α-humulene, which is the direct precursor of zerumbone. Yu et al. (2008) showed that ZSS1 produces α-humulene as its main product when expressed and tested in heterologous systems (Yu et al., 2008). This confirmed that ZSS1 controls the first committed step in zerumbone biosynthesis.

The discovery of ZSS1 was very important because it connected the basic terpenoid precursor (FPP) to zerumbone formation. Before this, the enzyme responsible for making the carbon skeleton of zerumbone was not known. Since terpene synthases decide the basic structure of terpenoids, they are very important in metabolic engineering. By using or modifying these enzymes, scientists can redirect metabolic flow toward desired products. Therefore, ZSS1 became the key starting point for rebuilding the zerumbone pathway in other organisms like yeast.

Cytochrome P450 Enzymes

After ZSS1 was identified, later studies focused on the next step in the pathway, which involves oxidation reactions (Yu et al., 2011). Cytochrome P450 enzymes (CYPs) are important because they add oxygen atoms to hydrocarbon molecules and help form more complex natural products.

In *Z. zerumbet*, CYP71BA1 was identified as a key enzyme in zerumbone biosynthesis. Yu et al. (2011) showed that CYP71BA1 converts α-humulene into 8-hydroxy-α-humulene through a hydroxylation reaction. This step is important because it modifies the simple hydrocarbon structure into an oxygenated intermediate that is closer to zerumbone.

However, P450 enzymes are known to be difficult to use in metabolic engineering. This is because plant P450s are usually attached to membranes, require special electron transfer partners, and depend on cofactors such as NADPH (Zhao et al., 2025). In microbial hosts, these enzymes often work inefficiently due to poor electron transfer and low catalytic efficiency. As a result, they often become a bottleneck in engineered pathways. This problem is not only seen in zerumbone production but also in other terpenoid systems such as artemisinin and nootkatone which will be discussed more ahead (Li et al., 2016; Ro et al., 2006; Zhang et al., 2018).

ZSD1 Enzymes

The final step in zerumbone biosynthesis was clarified with the identification of ZSD1, a short-chain dehydrogenase/reductase enzyme. ZSD1 converts the oxygenated intermediate (such as 8-hydroxy-α-humulene) into zerumbone through oxidation reactions (Okamoto et al., 2011). The discovery of ZSD1 completed the core biosynthetic pathway of zerumbone.

The identification of ZSD1 was very important for synthetic biology because it provided the final enzymatic step needed for full zerumbone production. Later studies also showed that improved versions of ZSD1 can increase zerumbone yield, making it an important target for metabolic engineering (Zhang et al., 2018). Overall, the discovery of ZSS1, CYP71BA1, and ZSD1 transformed zerumbone from a plant metabolite into a synthetic biology target that can be produced using engineered microbial systems (Zhang et al., 2018).

Synthetic Biology Approaches for Zerumbone Production

Reconstruction of the pathway in yeast

A major milestone in zerumbone synthetic biology was the successful reconstruction of its biosynthetic pathway in the yeast *Saccharomyces cerevisiae* (Zhang et al., 2018). In this study, researchers achieved the first de novo microbial production of zerumbone by introducing the key plant genes *ZSS1*, *CYP71BA1*, and *ZSD1* into a yeast host, together with a compatible cytochrome P450 reductase (*At*CPR1). This allowed yeast cells to convert basic carbon sources into zerumbone through a complete engineered pathway.

In this engineered system constructed by Zhang et al. (2018), the yeast mevalonate (MVA) pathway supplied the precursor farnesyl pyrophosphate (FPP). ZSS1 then converted FPP into α-humulene, which was further modified by CYP71BA1 with the help of *At*CPR1 to produce oxidized intermediates. Finally, ZSD1 catalyzed the last step to form zerumbone. This study demonstrated that complex plant natural product pathways can be functionally reconstructed in microbial hosts, making it possible for zerumbone as the target for industrial biotechnology.

Metabolic engineering strategies

To improve zerumbone production in yeast, several metabolic engineering strategies were applied to increase precursor supply and improve pathway efficiency. One key strategy was the overexpression of tHMG1, which encodes a rate-limiting enzyme in the mevalonate pathway (Yang et al., 2026; Zhang et al., 2018). This increased the

overall production of isoprenoid precursors, especially farnesyl pyrophosphate (FPP), which is essential for downstream zerumbone biosynthesis.

Another important strategy involved engineering of ERG20, the enzyme responsible for FPP synthesis (Zhang et al., 2018). Modifying ERG20 activity helped increase the pool of FPP available for conversion into α-humulene by ZSS1 (Yang et al., 2026; Zhang et al., 2018). In addition, regulation of ERG9, which competes for FPP by directing it toward sterol biosynthesis, was used to reduce carbon loss into unwanted pathways.

Together, these strategies redirected metabolic flux toward α-humulene production and improved the efficiency of the engineered pathway. In general, metabolic flux toward zerumbone was enhanced by strengthening the endogenous mevalonate pathway and reducing competing sterol biosynthesis routes, allowing more carbon to be channelled toward the desired product.

Challenges

Despite successful reconstruction of the zerumbone pathway, several challenges still limit high-level production. One major limitation is the relatively low zerumbone yield compared to other microbial terpenoid systems. Although production has been demonstrated, the yield remains substantially lower than many commercially successful sesquiterpenoid production systems, indicating that further pathway optimization is required (Zhang et al., 2018). Another major challenge is the limitation of cytochrome P450 enzymes. CYP71BA1 requires electron transfer partners and cofactors to function efficiently, and its activity is often reduced in microbial hosts due to poor coupling efficiency and membrane-associated expression issues (Li et al., 2022; Meng et al., 2020; Zhang et al., 2018; Zhao et al., 2025). This makes the oxidation step a bottleneck in the overall pathway.

In addition, product toxicity can affect yeast cell growth, especially when intermediate or final terpenoids accumulate at high levels in the cell membrane (Zhang et al., 2018). Increasing precursor availability alone is insufficient to maximize zerumbone production. Enhanced FPP flux may lead to α-humulene accumulation when downstream oxidation steps catalyzed by CYP71BA1 become rate-limiting, highlighting the importance of pathway balancing during microbial engineering (Yang et al., 2026). Finally, cofactor imbalance, particularly limited NADPH availability, can further restrict P450 activity and reduce overall pathway efficiency (Yang et al., 2026; Zhang et al., 2018).

Overall, these challenges highlight that while zerumbone production in yeast is feasible, significant optimization is still required to achieve industrial-level production. Similar challenges have been addressed in artemisinin, nootkatone, and patchoulol engineering through precursor enhancement, enzyme optimization, and pathway balancing, suggesting that these strategies may also improve future zerumbone production.

Other Engineered Sesquiterpenoids: The Guidelines

Artemisinin: The Benchmark of Plant Synthetic Biology

Artemisinin is one of the most successful examples of plant natural product production through synthetic biology. Originally obtained from *Artemisia annua*, artemisinin is an important antimalarial compound. Due to limitations in plant cultivation and extraction, researchers developed engineered *Saccharomyces cerevisiae* strains capable of producing artemisinic acid, a direct precursor of artemisinin (Ro et al., 2006). The importance of developing sustainable production platforms is reflected in the growing global demand for artemisinin. The global artemisinin market was valued at approximately USD 3.86 billion in 2023 and is projected to grow through 2030 (Yang et al., 2026). This growth is driven primarily by the continuing need for malaria treatment, with hundreds of millions of malaria cases reported annually, as well as increasing interest in potential applications of artemisinin and its derivatives in oncology and immunology (Li et al., 2016; Li et al., 2022; Yang et al., 2026).

Nootkatone

Nootkatone is a high value sesquiterpenoid responsible for the characteristic aroma of grapefruit. Like zerumbone, its biosynthesis involves oxidation reactions catalyzed by cytochrome P450 enzymes (Meng et al., 2020). Because of this, nootkatone production has been widely used as a model for oxidative pathway engineering. In addition to its commercial value in the flavour and fragrance industries, (+)-nootkatone has attracted considerable attention due to its reported therapeutic potential, including anticancer, antiplatelet aggregation, antimicrobial, and anti-inflammatory activities. These biological properties make nootkatone a promising pharmaceutical precursor and further increase interest in developing efficient microbial production systems.

Patchoulol

Patchoulol is a commercially important sesquiterpenoid that is widely used in the cosmetic, and premium perfume industries. Considerable progress has been made in producing patchoulol using engineered microbial systems. Successful production has been demonstrated in several microbial hosts, including *Escherichia coli*, *Pichia pastoris*, *Saccharomyces cerevisiae*, and *Yarrowia lipolytica (Yang et al., 2026)*. Through optimization of precursor supply, engineering of the mevalonate pathway, and improved terpene synthase expression, researchers have achieved substantially higher product titres than many other sesquiterpenoid production systems. Advanced metabolic engineering strategies such as acetyl-CoA optimization and pathway compartmentalization have further enhanced microbial patchoulol production (Li et al., 2022; Yang et al., 2026)

Discussion

Few key approaches that can be adapted such as increasing precursor supply by engineering the mevalonate pathway to boost farnesyl pyrophosphate (FPP) levels, which has been widely used in microbial terpenoid production (Li et al., 2016; Meng et al., 2020; Zhang et al., 2018). In addition, promoter engineering can help control the expression of *ZSS1*, *CYP71BA1*, and *ZSD1* so that metabolic flux is more balanced across the pathway (Li et al., 2022; Yang et al., 2026). However, experience from other terpenoid systems shows that introducing pathways alone is not enough for high production (Li et al., 2022; Yang et al., 2026). In nootkatone production, improving cytochrome P450 activity and electron transfer is essential to overcome oxidation

limits. In patchoulol production, metabolic balance is important because higher precursor levels do not always lead to higher final product if downstream steps are slow.

Artemisinin, nootkatone, and patchoulol highlight different aspects of successful sesquiterpenoid engineering. Artemisinin demonstrates the importance of increasing precursor supply through mevalonate pathway engineering, while nootkatone emphasizes the need to overcome cytochrome P450 bottlenecks. Patchoulol production further shows that pathway balancing is essential to achieve high product yields. Together, these systems provide a useful framework for future zerumbone engineering, where improvements in precursor supply, CYP71BA1 activity, and metabolic flux balance are likely required to increase production.

Future Perspectives

Protein Engineering

Protein engineering offers a promising strategy for improving zerumbone production. Based on Yang et al. (2026), directed evolution and site-directed mutagenesis can be used to modify enzymes such as ZSS1 and ZSD1 by changing specific amino acids involved in substrate binding and catalysis. These modifications may improve enzyme activity, stability and product formation. Similar approaches have previously proven effective in other terpenoid systems (Yang et al., 2026). For example, the mutant enzyme *Ph*CCD1(K164L) significantly improved β-ionone production in *Yarrowia lipolytica* by increasing the conversion efficiency of pathway intermediates. For zerumbone biosynthesis, engineering CYP71BA1 may improve oxidation efficiency, reduce α-humulene accumulation, and overcome bottlenecks caused by slow electron transfer. In addition, random mutagenesis and rational protein design could be used to generate new enzyme variants with improved performance or altered product specificity.

CRISPR-Based Metabolic Engineering

CRISPR-based genome editing provides powerful tools for optimizing microbial production pathways (Li et al., 2022; Yang et al., 2026). This technology enables the precise integration of biosynthetic genes into microbial genomes and allows fine-tuning of gene expression levels. For example, CRISPR/Cas9 has been used to integrate heterologous carotenoid biosynthetic genes into *Saccharomyces cerevisiae*, resulting in increased β-ionone production through enhancement of the mevalonate pathway and optimized gene expression strategies (Yang et al., 2026). Similar approaches could be applied to zerumbone biosynthesis by increasing the expression of key pathway genes such as *ZSS1, CYP71BA1,* and *ZSD1.* CRISPR-based engineering could also be used to modify *ERG20* to increase the supply of farnesyl diphosphate (FPP) and regulate *ERG9* to reduce carbon flux toward competing sterol biosynthesis pathways, thereby directing more precursors toward zerumbone production (Yang et al., 2026; Zhang et al., 2018). Furthermore, dynamic regulation strategies may help balance cell growth and product formation by adjusting pathway activity according to cellular conditions (Li et al., 2022).

AI-Guided Enzyme Design

Recent advances in artificial intelligence have created new opportunities for enzyme engineering and pathway optimization (Li et al., 2022). AI-based protein structure

prediction tools can help researchers understand enzyme function and identify potential mutation sites before conducting laboratory experiments. This can reduce the time and cost required for enzyme development. In addition, machine learning models can be used to predict pathway bottlenecks, optimize gene expression levels, and identify engineering strategies that improve product yield (Yang et al., 2026). These tools may accelerate the development of more efficient zerumbone-producing strains.

Sustainable Biomanufacturing

Microbial production offers a sustainable alternative to conventional cultivation and extraction of *Zingiber zerumbet*. Unlike field cultivation, microbial fermentation requires less land, can be completed within a shorter time, and can be scaled up according to industrial demand. Production can also be carried out under controlled conditions, reducing the effects of environmental factors and allowing continuous monitoring and optimization. As interest in zerumbone continues to grow due to its reported anticancer, anti-inflammatory, antimicrobial, and antioxidant properties, sustainable biomanufacturing could provide a reliable source of this valuable compound. In addition to supporting pharmaceutical development, successful zerumbone production may create economic opportunities for countries in Southeast Asia where *Z. zerumbet* is widely distributed.

Conclusion

Advances in elucidating the zerumbone biosynthetic pathway have transformed *Zingiber zerumbet* from a medicinally important species into a promising resource for synthetic biology-driven production of high-value natural products. Identification of key enzymes, including ZSS1, CYP71BA1, and ZSD1, has enabled the reconstruction of the pathway in microbial hosts and established the foundation for sustainable zerumbone production. Therefore, future progress should depend more on improving the performance of the existing pathway. Advances in integrating protein engineering to enhance enzyme activity, systems metabolic engineering to optimize carbon flux, and process optimization will be essential for increasing zerumbone yield. Lessons learned from the microbial production of artemisinin, patchoulol, and nootkatone provide useful strategies for future zerumbone engineering. Together with advances in synthetic biology and omics technologies, these approaches will help make zerumbone production more efficient and commercially viable.

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