Revolution of vitamin E production by starting from microbial fermented farnesene to isophytol

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GRAPHICAL ABSTRACT

PUBLIC SUMMARY
- The traditional chemical synthesis of vitamin E is complex and could be explosive
- An innovative way to synthesize isophytol from biofermented farnesene is established
- This process is safer and cheaper, changing the production and marketing of vitamin E
- Co-production of β-farnesene and lycopene improves the competitiveness of this process
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ABSTRACT

Vitamin E is one of the most widely used vitamins. In the classical commercial synthesis of vitamin E (\(\alpha\)-tocopherol), the chemical synthesis of isophytol is the key technical barrier. Here, we establish a new process for isophytol synthesis from microbial fermented farnesene. To achieve an efficient pathway for farnesene production, Saccharomyces cerevisiae was selected as the host strain. First, \(\beta\)-farnesene synthase genes from different sources were screened, and through protein engineering and system metabolic engineering, a high production of \(\beta\)-farnesene in S. cerevisiae was achieved (55.4 g/L). This farnesene can be chemically converted into isophytol in three steps with approximately 92% yield, which is economically equal to that from the best total chemical synthesis. Furthermore, we co-produced lycopene and farnesene to reduce the cost of farnesene. A factory based on this new process was successfully operated in Hubei Province, China, in 2017, with an annual output of 30,000 tons of vitamin E. This new process has completely changed the vitamin E market due to its low cost and safety.

INTRODUCTION

Vitamin E is the general term for tocopherols and tocotrienols, essential fat-soluble vitamins for humans.1 It has a wide range of physiological functions based on its antioxidation properties, including improving the body’s immunity and fertility, possessing anti-cancer and anti-inflammatory properties, heart protection, nerve protection, and other functional characteristics.2-5 Thus, it is widely used in feed additives, medicine, food, cosmetics, and other fields. Together with vitamins C and A, it is listed as one of the three pillar products of the vitamin series.

Vitamin E includes natural and synthetic vitamin E. For industrial production, natural vitamin E is mainly extracted from vegetable oil deodorizer distillate and other plants rich in vitamin E.6 Although this process preserves its natural activity, the low yield, complex extraction and purification processes, and dependence on the climate and culture make it difficult to meet the market demand. Only 20% of vitamin E comes from plant extraction.6 With the development of biotechnology, the biosynthetic pathway of vitamin E has been elucidated.7,8 In plants, vitamin E biosynthesis is initiated by homogentisate synthetized by the shikimate pathway and various hydrophobic polyprenyl pyrophosphates that determine the final type of vitamin E. Geranylgeranyl pyrophosphate (GGPP) derived from the 2C-methyl-D-erythritol-4-phosphate pathway is the precursor for tocotrienol, and phytyl pyrophosphate obtained from GGPP reduction or chlorophyll degradation followed by phosphorylation is used for tocopherol biosynthesis (Figure 1A).\(^{7,8}\) Researchers have attempted to increase the vitamin E content of plants by pathway engineering. Overexpression of barley homogentisate geranylgeranyl transferase, which catalyzes the committed step of tocotrienol biosynthesis in Arabidopsis thaliana leaves, resulted in a 10- to 15-fold increase in tocotrienol and tocopherol levels,\(^{7,8}\) and its overexpression in soybean seeds has also sufficiently increased tocochromanol levels by 8- to 10-fold.\(^{11}\) In Nicotiana benthamiana, the transient expression of tocopherol cyclase and homogentisate phytyltransferase from A. thaliana has increased \(\alpha\)-tocopherol levels by 11.3 fold.\(^{12}\) However, it is still difficult to meet the increasing market demand for vitamin E.

Currently, 80% of commercial vitamin E comes from chemical synthesis, and it is mainly consumed in the feed industry. Classical synthesis of vitamin E (\(\alpha\)-tocopherol) is via the condensation of isophytol and trimethylhydroquinone, and the production capacity and cost largely depend on the production of these two materials. Due to the increasing demand for vitamin E, many different synthetic routes have been developed and optimized to synthesize vitamin E precursors. The \(\alpha\)-tocopherol and pseudoionone processes are the main synthetic routes for synthesizing trimethylhydroquinone and isophytol,\(^{13,14}\) respectively. By 2017, the classical processes used to synthesize isophytol and trimethylhydroquinone were relatively mature, and the entire market price of vitamin E was approximately US$220/kg based on the use of classical technology.

The rapid development of metabolic engineering and synthetic biology has made the microbial cell factory a game changer in chemical production.14 The efficient production of various compounds, such as the anti-cancer drug taxol precursor taxadene,\(^{15}\) anti-malarial drug precursor artemisinic acid,\(^{16-17}\) advanced biofuel short-chain alkanes,\(^{18}\) fatty acids,\(^{19,20}\) and biopolymer polyhydroxyalkanoates,\(^{21}\) has demonstrated the advantages and potential of microbial fermentation in terms of cost effectiveness. Some studies have also achieved the heterologous synthesis of vitamin E in microorganisms. Albermann et al. heterologously expressed vitamin E biosynthesis genes from Synecochysis and A. thaliana in Escherichia coli, resulting in 15 \(\mu\)g/g \(\delta\)-tocotrienols.\(^{22}\) The de novo production of \(\delta\)-tocotrienol from glucose with a titer of 4.1 mg/L in fed-batch fermentation was achieved in S. cerevisiae.\(^{23}\) Furthermore, Shen et al. comprehensively optimized the vitamin E heterologous pathway and the endogenous precursor pathway, designed a cold-shock-triggered temperature control system, and successfully constructed high-tocotrienol-producing S. cerevisiae with the production of 320 mg/L.\(^{24}\) Although these studies have laid the foundation for the production of vitamin E by microbial fermentation, continued efforts are warranted to ensure the possibility of commercial production.

In 2014, our group efficiently synthesized farnesene by refactoring the mevalonate (MVA) pathway in E. coli.\(^{25}\) In the same year, Nenter & Co. innovated the synthesis of trimethylhydroquinone and was eagerly looking for a way to break through the isophytol synthesis process.\(^{26}\) Upon noticing this need, we proposed a new innovative process of using biofermented farnesene as a substrate to synthesize isophytol to subvert the isophytol synthesis pathway (Figure 1C). Here, we provide a retrospect on how this disruptive new approach was established in detail.

RESULTS

Evaluating the possibility of different methods to generate isophytol

We evaluated potential routes for synthesizing vitamin E based on cost, safety, and efficiency. Previously, vitamin E could be obtained only by plant extraction and chemical synthesis. Chemical synthesis of vitamin E has great advantages and occupies the entire market in the feedstock industry. It is achieved by condensing isophytol and trimethylhydroquinone (Figure 1D). The synthesis of trimethylhydroquinone is relatively easy, whereas the chemical synthesis of isophytol is difficult. The pseudoionone process is the classical process used to prepare isophytol and is initiated by the condensation of citral and acetone to pseudoionone.\(^{27}\) and isophytol can be obtained by hydrogenation, ethynylation, and condensation of pseudoionone in seven steps (Figure 1B).\(^{28}\) This process involves a multistep alkyne reaction under high pressure, with high requirements for equipment and safety.

Synthetic biology is a highly safe procedure used to produce bulk chemicals with a low environmental burden. However, several difficulties are associated with the microbial synthesis of vitamin E by heterologous expression of synthetic genes from plants or other suitable resources. First, this biosynthesis pathway needs to introduce multiple heterologous genes with many uncertainties regarding their protein expression and catalytic efficiency. Meanwhile, achieving a high titer of intracellular products with the relatively higher molecular weights and cell toxicity is difficult.\(^{29,30}\) Most importantly, the cost of the chemical synthesis of vitamin E is approximately US$10/kg, thus, it is...
to isophytol can simplify the synthesis process and reduce the use of explosive.

In this process, the three-step reaction via the conversion of farnesene was then converted to isophytol by hydrogenation and addition reactions (Figure 1C). In the past 10 years, most groups considered that farnesene was only the precursor for jet fuel. However, we previously conceived a process to obtain farnesyl acetone via farnesene, which was then converted to isophytol by hydrogenation and addition reactions (Figure 1C). In this process, the three-step reaction via the conversion of farnesene to isophytol can simplify the synthesis process and reduce the use of explosive raw materials.

Therefore, this new method for synthesizing isophytol via farnesene obtained by microbial fermentation could be promising in producing vitamin E.

Evaluation of α- or β-farnesene to isophytol

Farnesene includes α- and β-farnesene, and discrepancies in their structures may influence the efficiency of conversion to isophytol. To validate our proposed process for the synthesis of isophytol using farnesene as a substrate, E. coli F47 with nearly 2 g/L production reported in previous work, was fermented for α-farnesene preparation; after 100 h of shake-flask fermentation, the strain was extracted with hexane/ethyl acetate (4:1) and then separated using silicagel column chromatography. α-Tocopherol was isolated and used for the synthesis of vitamin E. Therefore, this new method for synthesizing isophytol via farnesene obtained by microbial fermentation could be promising in producing vitamin E.
reaction was performed at room temperature and atmospheric pressure. Hydrogen without heating and high pressure; it was safe and energy efficient. Isophytol (3) was obtained in 99% yield by the reaction of hexahydrofarnesylacetone (2) with vinyl magnesium chloride in tetrahydrofuran under an N₂ atmosphere (supplemental materials and methods).

This three-step reaction can be used to realize the conversion of β-farnesene obtained by microbial fermentation to isophytol, with an overall yield of 92%. This result demonstrates the excellent potential of this new process to replace the traditional process of isophytol synthesis. In 2017, the process from farnesene to isophytol and further to vitamin E was put into production by Nenter & Co. in Jingzhou, Hubei Province, China, and an annual output of 30,000 tons of vitamin E has since been achieved.

Efficient microbial synthesis of farnesene

The conversion of farnesene to isophytol was confirmed. However, whether this process is revolutionary depends on the yield, titer, and productivity of farnesene. Therefore, the efficient synthesis of farnesene is very important, and in this study, we aimed to achieve high production of β-farnesene.

Chassis selection. *E. coli* is easily infected by phages; it is not very good for biomass use and is not tolerant to high farnesene concentrations. Compared with *E. coli*, *S. cerevisiae* is generally regarded as safe and robust under harsh industrial conditions,²⁰ and it was thus selected as the chassis strain. Farnesyl diporphosphate (FPP) is derived from the MVA pathway for sesquiterpene synthesis, and the weak synthetic capacity of the native MVA pathway in yeast limits the production of sesquiterpenes.⁴¹,⁴² To achieve high production, the flux of the MVA pathway needs to be increased.⁴³ We used the CRISPR-Cas9 system for MVA pathway engineering to avoid a lack of markers. First, the Cas9 gene from the type II bacterial CRISPR system of *Streptococcus pyogenes* was integrated into *CEN.PK 2-1D to generate JCR1.* Then ERG8, THMG1 (two copies) and ERG12, MVK1, IDT1, and ERG13 under the control of P₅₆₃₅₋₅₆₃₁₃ and P₅₆₃₇ were overexpressed in the JCR1 genome via three integration steps to generate JCR27. Our group successfully produced sesquiterpene guaia-6,10(14)-dien-3-ol in a high titer in a previous study based on this precursor-providing platform;⁴⁴ therefore, strain JCR27, with overexpression all the MVA pathway genes, was used as the platform strain.

Selection of farnesene synthase. Farnesene is present in various plants and varies in content. For gene screening, we selected *Mac-bFS* from *Matricaria chamomilla var. recutita* (GenBank: AIG92847.1)²⁵ and *Aa-bFS* from *Artemisia annua* (GenBank: AAX39387.1),²⁶ both of which have been isolated and characterized. To expand the screening ranges for β-farnesene synthases, we also selected several putative β-farnesene synthases from the NCBI database, including predicted β-farnesene synthases from *Helianthus annuus* (GenBank: KAF5755268), *Tanacetum cineranilium* (GenBank: GEU62861.1), *Cynara cardunculus* (GenBank: XP_024994934.1), and *Lactuca sativa* (GenBank: XP_023749309.1). ERG20 to improve FPP flux and β-farnesene synthase under the control of P₅₆₃₅₋₅₆₃₁₃ were overexpressed by episomal plasmids in the MVA-overexpressing strain JCR27, resulting in strains BFS1 (Mac-bFS), BFS2 (Hca-bFS), BFS4 (Tac-bFS), BFS5 (Cyc-bFS), BFS6 (Las-bFS), and BFS7 (Aa-bFS). BFS1 produced 203 ± 3.8 mg/L β-farnesene after 72 h of incubation, which was higher than that produced by BFS7 (184 ± 5.72 mg/L) (Figure 2B), but strains BFS2, 4, 5, and 6 failed to detect the target product in vivo, indicating that the activity of β-farnesene synthase from *M. chamomilla var. recutita* was higher in *S. cerevisiae* than that of others. Therefore, Mac-bFS was chosen for use in the next construction.

Farnesene synthase engineering. The catalytic efficiency of farnesene synthase determines the flux from FPP to farnesene. Further enhancing the catalytic activity of farnesene synthase can increase the titer of farnesene. Directed evolution is a powerful strategy used to change the catalytic activity of enzymes, and it is possible to obtain mutants with improved performance based on a suitable targeted screening method.⁴⁷,⁴⁸ In this study, directed evolution was employed to improve the Mac-bFS activity. The crystal structure of farnesene synthase from *M. chamomilla var. recutita* has not been solved yet; therefore, a random approach was adopted to mutate Mac-bFS (Figure 3). To isolate improved farnesene synthase variants, we developed a simple high-throughput screening method to single out improved farnesene synthase variants that detect the content difference of farnesene in strains based on the vanillin-sulfuric acid method. The expression of wild-type farnesene synthases resulted in low absorbance, and mutants with an increased β-farnesene yield were selected. After further shake-flask fermentation, plasmid extraction, and sequencing, we identified mutations in pBFs9 (F11S, TTC-TCC), pBFs12 (M35T, ATG-ACC), pBFs15 (T319S, ACT-TCT), pBFs18 (I434T, ATT-ACT), and pBFs20 (I460V, ATC-GTC), and the production in these variants was 1.46-, 1.51-, 1.52-, 1.65-, and 2.84-fold, respectively, compared with that in BFS1 with wild-type β-farnesene synthase (Table S1).

The 3D structure of proteins, including those in BFS1 (wild type), BFS9 (F11S), BFS12 (M35T), BFS15 (T319S), BFS18 (I434T), and BFS20 (I460V), was simulated using SWISS-MODEL (https://swissmodel.expasy.org/), and the crystal structure of (+)-delta-cadinene synthase (PDB: 3G4D) was used as the template for wild-type and mutant modeling. The substrate FPP was docked to the simulated structures using AutoDock 4, and the binding site for the docking procedure was defined according to the crystal structure of another homologous sesquiterpene synthase (PDB: 4RNO). The docking parameters were set as default. The results showed that the distance between the five mutation sites and FPP was more than 5 Å. The docking results are shown in Figures S1–S6. In addition,
the docking binding energy between FPP and the enzymes showed that the five mutations had a stronger affinity with the substrate than the wild type (Table S1); therefore, we inferred that this might be the reason for the increase in activity. The beneficial effects of single mutations are often additive; therefore, we combined the improved mutation in Mac-bFS, and the best b-farnesene synthase variant BFS45 (F11S, M35T, T319S, I434T, I460V) improved the titer by approximately 2-fold.

Pathway engineering. The expression system of chromosomal integration is preferred over an episomal plasmid for industrial applications as it is more stable. Therefore, for the construction of producing strains, all modifications to the host strain were made by chromosomal integration. pBFS45 harboring the FPP synthase Erg20 and the best b-farnesene synthase variant was linearized and integrated into the JCR27 genome, and JVA122 was obtained with a b-farnesene titer of 437 mg/L (Figure 4).

To achieve commercially viable farnesene production, metabolic fluxes must be fine-tuned to maximize farnesene titers. The farnesene biosynthetic pathway can be divided into an upstream MVA pathway and a downstream farnesene synthetic pathway. We adopted a “push-pull” strategy to balance the upstream and downstream fluxes and tuned the copy number of the rate-limiting enzyme thMG1 in the upstream pathway and farnesene synthase in the downstream pathway to increase the production. b-farnesene production increased with the increase in copy number, and the titer in the b-farnesene-producing strain JVA139 with a copy number of 5 for Mac-bFS45 based on JCR27 was 791 mg/L (Figure 4). We further deleted GAL80 to constitutively express all heterologous genes under the control of galactose-inducible promoters, and the titer of the resulting strain JVA140 reached up to 736 mg/L, without the addition of galactose, which would thus reduce the fermentation cost.

Farnesene overproduction by high-cell-density fermentation

To test the performance of JVA140 for farnesene production, we conducted fed-batch fermentation in 1 L bioreactors. The fermentation media are listed in the supplemental materials and methods. Under two-stage
fermentation, biomass accumulated quickly in the first stage, and the products accumulated after glucose exhaustion. At the beginning of fermentation, the glucose concentration was 40 g/L; it was then consumed due to cell growth, and when it was close to 1 g/L, feeding solution I was added at a controlled feeding rate to sustain cell growth and to maintain the glucose concentration at approximately 0–1 g/L. Furthermore, 10% v/v isopropyl myristate was added to cultures at 24 h to reduce product loss. When cell growth slowed, feeding solution II was used for product accumulation, and the ethanol concentration was maintained at 5–10 g/L. JVA140 achieved a titer of 55.4 g/L β-farnesene and a yield of 6.5% grams of farnesene per gram of glucose after 126 h cultivation (Figure 5A).

The new process of vitamin E synthesis using farnesene was considered to have a competitive cost advantage over that of chemical vitamin E synthesis only when the microbial fermentation cost for farnesene was estimated to be lower than US$6/kg. However, the carbon cost of JVA140 microbial fermentation for farnesene is approximately US$6/kg. As the titer by Amyris can reach 130 g/L for farnesene is approximately US$60/kg. The process is thus illustrative of the success of using synthetic biology and the production safety of this process is greatly improved compared with that of previous methods. In addition, carbon dioxide emissions are reduced by 50%. The process can be scaled up to 1 L fed-batch fermentation, and the β-farnesene and lycopene titers reached 616 and 419 mg/L (6.5 mg/g dry cell weight (DCW)), respectively, at 120 h (Figure 5B). The overall yield was 0.16% grams of products per gram of glucose, with a carbon cost of approximately US$600/kg lycopene plus 1.5 kg β-farnesene; although the cost is still high, the market price of lycopene is optimal combination of PaCrtE from Pantoaea ananatis, PagCrtI from P. agglomerans, and BtCrtI from B. trispora was obtained, and thHM1, CrtE, and CrtI were found to be the rate-limiting enzymes in the lycopene biosynthesis pathway.30 For lycopene overproduction in the farnesene-producing strain, ribosomal DNA multi-copy integration of PaCrtE, BtCrtI, and PagCrtI, thHM1 was implemented in JVA139 to obtain mutants. Red colonies were selected to integrate URA3 and delete GAL80 to generate a prototrophic and non-galactose-inducible strain. JZL32 can produce 106 ± 6 mg/L β-farnesene and 63 ± 5 mg/L lycopene after 72 h incubation. It was evident that the farnesene titer was reduced significantly with lycopene production, mainly because of the competition for the common precursor FPP. JZL32 was subjected to 1 L fed-batch fermentation, and the β-farnesene and lycopene titers reached 661 and 426 mg/L at 120 h (Figure 5B). The overall yield was 0.16% grams of products per gram of glucose, with a carbon cost of approximately US$600/kg lycopene plus 1.5 kg β-farnesene; although the cost is still high, the market price of lycopene is

Co-production of farnesene and lycopene

We envisaged the introduction of high-value-added compounds based on farnesene-producing strains to reduce the microbial synthesis cost. Lycopene is an excellent antioxidant with favorable physiological effects and is widely used in the health care product market; the demand and market for lycopene have increased dramatically. Our group has previously conducted a considerable amount of research on the microbial synthesis of lycopene,29–31,32 and we have a relatively solid experimental foundation; therefore, we considered introducing the lycopene synthesis pathway into the farnesene-producing strain. In a previous study, the near US$2,000/kg, which can cover the fermentation cost. Therefore, microbial fermentation for farnesene accompanied by lycopene is almost a cost-free process, and only the purification cost needs to be considered.

DISCUSSION

Vitamin E is one of the most in-demand vitamin products in the global market. At present, the global annual demand is approximately 80,000 tons. The price of vitamin E fluctuates with the price of raw materials. In this study, we proposed a new process for synthesizing the vitamin E precursor isophytol. The process uses biosynthetic farnesene as the substrate to synthesize isophytol prior to synthesizing vitamin E. We first converted farnesene to isophytol, and the three-step reaction can realize a traditional seven-step process. The new process is efficient, is safe, and uses fewer explosive raw materials. Based on this new process proposed and verified in this study, a plant with 30,000 ton capacity per year was established in Jinzhous, Hubei Province, through the efforts of Nenter & Co. (200 km from Wuhan University) and has been in production since 2017. The process used is conducive for stabilizing the supply and price of vitamin E. As such, the market price of vitamin E has dropped from US$20/kg to approximately US$10/kg. This process combines synthetic biology with chemical synthesis, and therefore, the production safety of this process is greatly improved compared with that of previous methods. In addition, carbon dioxide emissions are reduced by 50%. The process is thus illustrative of the success of using synthetic biology to improve the classical chemical process.

Currently, 20% of the global vitamin E yield is produced using this process, and Nenter & Co. uses β-farnesene provided by the factory of Amyris in Brazil due to the company’s lower-cost fermented β-farnesene. Further improving the titer and yield of the microbial fermentation of farnesene would make this process more competitive. We developed our method by first achieving the overproduction of β-farnesene in S. cerevisiae through systematic metabolic engineering; however, the titer and yield were not sufficient for the method to replace the use of chemical synthesis. The lycopene synthesis pathway was then introduced into the high-production strain of farnesene, and this realized the “cost-0” synthesis of

Figure 4. β-farnesene production by fine-tuning FS and thHM1 Effects of the expression of different copy numbers of Mac-bFS (F11S, M35T, T319S, I43AT, I460V) and thHM1 on β-farnesene production in S. cerevisiae. ** means wild-type β-farnesene Mac-bFS. Error bars indicate the standard deviations of three biological replicates.
farnesene accompanied by the production of the high-value-added antioxidant lycopene by microbial fermentation. However, there was a significant reduction in the production of farnesene when producing it together with lycopene due to precursor competition, which may increase the difficulty of extraction. In the future, we can further optimize the microbial fermentation pathway and reduce the synthetic cost of farnesene in two ways: (1) by rewiring the central carbon metabolism to improve precursor supply, downregulating the competitive pathway, and prolonging the period of high productivity by introducing a genetic circuit that can be used to improve the titer and yield of farnesene and (2) by improving the microbial fermentation process of farnesene production by using products in the non-MVA pathway, such as inositol and succinic acid, which may be promising to reduce costs. It is anticipated that with further advances, the remaining 80% of the vitamin E capacity may be replaced by this process in the future.

REFERENCES

1. Peh, H.Y., Tan, W.S., Liao, W., and Wong, W.S. (2016). Vitamin E therapy beyond cancer: tocopherol versus tocotrienol. Pharmacol. Theor. 162, 152–169.
2. Lee, G.Y., and Han, S.N. (2018). The role of vitamin E in immunity. Nutrients 10, 1614.
3. Mohd Mutalip, S.S., Ab-Rahim, S., and Rajikin, M.H. (2018). Vitamin E as an antioxidant in female reproductive health. Antioxidants 7, 22.
4. Aqlahtani, S., and Kaddoumi, A. (2015). Vitamin E transporters in cancer therapy. AAPS J. 17, 313–322.
5. Li, M., Pham, P.J., Pittman, C.U., et al. (2008). Selective solid-phase extraction of alpha-tocopherol by functionalized ionic liquid-modified mesoporous SBA-15 adsorbent. Anal. Sci. 24, 1245–1250.
6. Ogbonna, J.C. (2009). Microbiological production of tocopherols: current state and prospects. Appl. Microbiol. Biotechnol. 84, 217–223.
7. Cahoon, E.B., Hall, S.E., Ripp, K.G., et al. (2003). Metabolic redesign of vitamin E biosynthesis in plants to tocotrienol production and increased antioxidant content. Nat. Biotechnol. 21, 1082–1087.
8. Zeng, Z., Han, N., Lu, C., et al. (2020). Functional dissection of HGG7 and HPT in barley vitamin E biosynthesis via CRISPR/Cas9-enabled genome editing. Ann. Bot. 126, 929–942.
9. Mene-Saffrané, L. (2017). Vitamin E biosynthesis and its regulation in plants. Antioxidants 7, 2.
10. Muñoz, P., and Munne-Bosch, S. (2019). Vitamin E in plants: biosynthesis, transport, and function. Trends Plant Sci. 24, 1040–1051.
11. Konda, A.R., Nazarenus, T.J., Nguyen, H., et al. (2020). Metabolic engineering of soybean seeds for enhanced vitamin E tocotrienol content and effects on oil antioxidant properties in polysaturated fatty acid-rich germplasm. Metab. Eng. 57, 63–73.
12. Sathish, S., Preethy, K.S., Venkatesh, R., and Sathishkumar, R. (2018). Rapid enhancement of alpha-tocopherol content in Nicotiana benthamiana by transient expression of Arabidopsis thaliana tocopherol cyclase and homogentisate phytyl transferase genes. 3. Biotech. 8, 485.
13. Netscher, T. (2007). Synthesis of vitamin E. Vitam. Horm. 76, 155–202.
14. Ren, Y., Liu, S., Jin, G., et al. (2020). Microbial production of limonene and its derivatives: achievements and perspectives. Biotechnol. Adv. 44, 107628.
15. Ajikumar, P.K., Xiao, W.H., Tyo, K.E., et al. (2010). Isoprenoid pathway optimization for taxol precursor overproduction in Escherichia coli. Science 330, 70–74.
16. Ro, D.K., Paradise, E.M., Ouellet, M., et al. (2006). Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440, 940–943.
17. Paddon, C.J., Westfall, P.J., Pitera, D.J., et al. (2013). High-level semi-synthetic production of the potent antimalarial artesiminin. Nature 496, 528–532.
18. Choi, Y.J., and Lee, S.Y. (2013). Microbial production of short-chain alkanes. Nature 502, 571–574.
19. Yu, T., Zhou, Y.J., Huang, M., et al. (2018). Reprogramming yeast metabolism from alcoholic fermentation to lipogenesis. Cell 174, 1549–1558, e14.
20. Kim, H.M., Chae, T.U., Choi, S.Y., et al. (2019). Engineering of an oleaginous bacterium for the production of fatty acids and fuels. Nat. Chem. Biol. 15, 721–729.
21. Ma, H., Zhao, Y., Huang, W., et al. (2020). Rational flux-tuning of Halomonas bluephage for co-production of biopolymorphic PHB and ectoine. Nat. Commun. 11, 3313.
22. Albermann, C., Ghanegaonkar, S., Lemuth, K., et al. (2008). Biosynthesis of the vitamin E compound delta-tocotrienol in recombinant Escherichia coli cells. Chembiochem 9, 2524–2533.
23. Sun, H., Yang, J., Lin, X., et al. (2020). De novo high-titer production of delta-tocotrienol in recombinant Saccharomyces cerevisiae. J. Agric. Food Chem. 68, 7710–7717.
24. Chen, B., Zhou, P., Jiao, X., et al. (2020). Fermentative production of vitamin E tocotrienols in Saccharomyces cerevisiae under cold shock-triggered temperature control. Nat. Commun. 11, 5155.
25. Zhu, F., Zhong, X., Hu, M., et al. (2014). In vitro reconstitution of mevalonate pathway and targeted engineering of farnesene overproduction in Escherichia coli. Biotechnol. Bioeng. 111, 1396–1405.
26. Ma, T., Deng, Z., and Liu, T. (2020). The past and present of vitamin E. Synth. Biol. J. 1, 174–186.
27. Kustrowitski, P., Sukowska, D., Chmielarz, L., and Dziembaj, R. (2006). Aldol condensation of citral and acetone over mesoporous catalysts obtained by thermal and chemical activation of magnesium–aluminum hydrotalcite-like precursors. Appl. Catal. A. 302, 317–324.
28. Aec Chim Organ Biol (1967). Improvements in and Relating to Process of Preparing Isophytol, [P].
29. Mal, T., Shi, B., Ye, Z., et al. (2019). Lipid engineering combined with systematic metabolic engineering of Saccharomyces cerevisiae for high-yield production of lycopene. Metab. Eng. 52, 134–142.
30. Shi, B., Ma, T., Ye, Z., et al. (2019). Systematic metabolic engineering of Saccharomyces cerevisiae for lycopene overproduction. J. Agric. Food Chem. 67, 11148–11157.
31. Kang, W., Ma, T., Liu, M., et al. (2019). Modular enzyme assembly for enhanced cascade biocatalysis and metabolic flux. Nat. Commun. 10, 4248.
32. Li, L., Liu, Z., Jiang, H., and Mao, X. (2020). Biotechnological production of lycopene by microorganisms. Appl. Microbiol. Biotechnol. 104, 10307–10324.
33. Gutbrod, K., Romer, J., and Dörmann, P. (2019). Phytol metabolism in plants. Prog. Lipid Res. 74, 1–17.
34. Hunter, S.C., and Cahoon, E.B. (2007). Enhancing vitamin E in oilseeds: unraveling tocopherol and tocotrienol biosynthesis. Lipids 42, 1–108.
35. Liu, R., Zhu, F., Lu, L., et al. (2014). Metabolic engineering of fatty acyl-ACP reductase-dependent pathway to improve fatty alcohol production in Escherichia coli. Metab. Eng. 22, 10–21.
36. Wenning, L., Yu, T., David, F., et al. (2017). Establishing very long-chain fatty alcohol and wax ester biosynthesis in Saccharomyces cerevisiae. Biotechnol. Bioeng. 114, 1025–1035.
37. Hu, Y., Zhu, Z., Nielsen, J., and Siewers, V. (2018). Heterologous transporter expression for improved fatty alcohol secretion in yeast. Metab. Eng. 45, 51–58.
38. Morel, D. (1983). Process for the Selective Addition of a Compound Having an Activated Carbon Atom onto a Substituted Conjugated Diene. [P].
39. Bian, G., Hou, A., Yuan, Y., et al. (2018). Metabolic engineering-based rapid characterization of a sesquiterpene cyclase and the skeletons of fusariumdiene and fusagramineol from Fusarium graminearum. Org. Lett. 20, 1626–1629.
40. Lian, J., Si, T., Nair, N.U., and Zhao, H. (2014). Design and construction of acetyl-CoA overproduction pathway to improve fatty alcohol production in Saccharomyces cerevisiae. Metab. Eng. 24, 139–149.
41. Chen, H., Zhu, C., Zhu, M., et al. (2019). High production of valencene in Saccharomyces cerevisiae through metabolic engineering. Microb. Cell Fact. 18, 195.
42. Peng, B., Plan, M.R., Chrysanthopoulos, P., et al. (2017). A squalene synthase protein degradation method for improved sesquiterpene production in Saccharomyces cerevisiae. Metab. Eng. 39, 209–219.
43. Bian, G., Deng, Z., and Liu, T. (2017). Strategies for terpenoid overproduction and new terpenoid discovery. Curr. Opin. Biotechnol. 48, 234–241.
44. Siemon, T., Wang, Z., Bian, G., et al. (2020). Semisynthesis of plant-derived engelina A enabled by microbe engineering of guaia-6,10(14)-diene as building block. J. Am. Chem. Soc. 142, 2760–2765.
45. Son, Y.J., Kwon, M., Ro, D.K., and Kim, S.U. (2014). Enantioselective microbial synthesis of the indigenous natural product (-)-alpha-bisabolol by a sesquiterpene synthase from Chamomile (Matricaria recutita). Biochem. J. 463, 239–248.
46. Picaud, S., Brodeleur, M., and Brodelius, P.E. (2005). Expression, purification and characterization of recombinant (E)-beta-farnesene synthase from Artemisia annua. Phytochemistry 66, 961–967.
47. Bloom, J.D., Meyer, M.M., Meinhold, P., et al. (2019). Innovative strategies for enzyme engineering. Curr. Opin. Struct. Biol. 15, 447–452.
48. Arnold, F.H. (2019). Innovation by evolution: bringing new chemistry to life (Nobel Lecture). Angew. Chem. Int. Ed. Engl. 58, 14420–14426.
49. Leonard, E., Ajikumar, P.K., Thayer, K., et al. (2010). Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. Proc. Natl. Acad. Sci. U. S. A. 107, 13654–13659.
50. Flagella, D.B., Siewers, V., Huang, L., and Nielsen, J. (2009). Characterization of chromosomal integration sites for heterologous gene expression in Saccharomyces cerevisiae. Yeast 26, 545–551.
51. Meadows, A.L., Hawkins, K.M., Tsegaye, Y., et al. (2016). Rewriting yeast central carbon metabolism for industrial isoprenoid production. Nature 537, 694–697.
52. Zhu, F., Li, L., Fu, S., et al. (2015). Targeted engineering and scale up of lycopene overproduction in Escherichia coli. Process. Biochem. 50, 341–346.

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AUTHOR CONTRIBUTIONS
T.L. conceived the idea of farnesene conversion to isophytol and organized teams to complete the work. Z.Y. performed the microbial synthesis experiments. K.S. was responsible for the chemical synthesis. T.L., Z.Y., and K.S. performed data analysis and wrote the manuscript. T.L. and Z.Y. edited the manuscript. B.S., Y.H., T.M., Z.X., Z.K., B.H., X.L., M.H., and Z.T. performed some experiments. Z.D. participated in the coordination and drafted the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors have applied for a series patents based on this work.

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