



Production of borneol, camphor, and bornyl acetate using engineered *Saccharomyces cerevisiae*

Tominaga, Masahiro ; Kawakami, Kazuma ; Ogawa, Hiro ; Nakamura, Tomomi ; Kondo, Akihiko ; Ishii, Jun

(Citation)

Metabolic Engineering Communications, 20:e00259

(Issue Date)

2025-06

(Resource Type)

journal article

(Version)

Version of Record

(Rights)

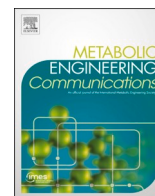
© 2025 The Authors. Published by Elsevier B.V. on behalf of International Metabolic Engineering Society.

This is an open access article under the Creative Commons Attribution 4.0 International license

(URL)

<https://hdl.handle.net/20.500.14094/0100495627>





Production of borneol, camphor, and bornyl acetate using engineered *Saccharomyces cerevisiae*

Masahiro Tominaga^{a,b,1}, Kazuma Kawakami^{b,1}, Hiro Ogawa^b, Tomomi Nakamura^{a,b}, Akihiko Kondo^{a,b,c,d}, Jun Ishii^{a,b,c,*}

^a Engineering Biology Research Center, Kobe University, 1-1 Rokkodai, Nada, Kobe, 657-8501, Japan

^b Graduate School of Science, Technology and Innovation, Kobe University, 1-1 Rokkodai, Nada, Kobe, 657-8501, Japan

^c Department of Chemical Science and Engineering, Faculty of Engineering, Kobe University, 1-1 Rokkodai, Nada, Kobe, 657-8501, Japan

^d Center for Sustainable Resource Science, RIKEN, 1-7-22 Suehiro, Tsurumi, Yokohama, 230-0045, Japan

ARTICLE INFO

Keywords:

Monoterpene
Borneol
Camphor
Bornyl acetate
Yeast

ABSTRACT

Microbial production of bicyclic monoterpenes is of great interest because their production primarily utilizes non-sustainable resources. Here, we report an engineered *Saccharomyces cerevisiae* yeast that produces bicyclic monoterpenes, including borneol, camphor, and bornyl acetate. The engineered yeast expresses a bornyl pyrophosphatase synthase from *Salvia officinalis* fused with mutated farnesyl pyrophosphate synthase from *S. cerevisiae* and two mevalonate pathway enzymes (an acetoacetyl-CoA thiolase/hydroxymethylglutaryl-CoA [HMG-CoA] reductase and an HMG-CoA synthase) from *Enterococcus faecalis*. The yeast produced up to 23.0 mg/L of borneol in shake-flask fermentation. By additionally expressing borneol dehydrogenase from *Pseudomonas* sp. TCU-HL1 or bornyl acetyltransferase from *Wurfbainia villosa*, the engineered yeast produced 23.5 mg/L of camphor and 21.1 mg/L of bornyl acetate, respectively. This is the first report of heterologous production of camphor and bornyl acetate.

1. Introduction

Bicyclic monoterpenoids, including borneol, camphor, and bornyl acetate, are used in traditional herbal medicine by leveraging their biological activities, which include anti-inflammatory, analgesic, antibacterial, antitumor, and anti-anxiety effects, and they are also used in fragrances and cosmetics (Ma et al., 2023). These monoterpenes are commercially available and can be extracted with high enantioselectivity from natural sources (e.g., *Cinnamomum burmanni* and *Blumea balsamifera* for borneol [Li et al., 2022], *Cinnamomum camphora* for camphor [Zhou and Yan, 2016], and *Amomum villosum*, *Inula graveolens*, and *Tetralinis articulata* for bornyl acetate [Zhao et al., 2023]). However, the supply chains of these products are unstable due to limited space for plant cultivation and low yield. Although racemic borneol and camphor can be chemically synthesized from α -pinene, a major constituent of turpentine oil (Ponomarev and Mettee, 2016), this process

produces a toxic by-product, isoborneol, which may cause serious side effects. Furthermore, chemical synthesis of these compounds uses harmful catalysts, such as heavy metals (Ponomarev and Mettee, 2016). Thus, alternative methods to sustainably produce these monoterpenes are needed.

Borneol, camphor, and bornyl acetate are naturally biosynthesized in the plants via the isoprenoid pathway, as follows (Fig. 1). Isoprenyl-pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) are produced via the mevalonate pathway and condensed into geranyl-pyrophosphate (GPP) by farnesyl pyrophosphate synthetase, Erg20p. GPP is then further circularized to bornyl-pyrophosphate (BPP) by BPP synthase (BPPS), and BPP is dephosphorylated into borneol. Borneol dehydrogenase (BDH) and bornyl acetyltransferase (BAT) convert the resultant borneol into camphor and bornyl acetate, respectively.

BPPSs are found in a diverse array of plants, including *B. balsamifera*, *C. burmanni*, *A. villosum*, *Lavandula angustifolia*, *Salvia officinalis*, and

Abbreviations: BAT, bornyl acetyltransferase; BDH, borneol dehydrogenase; BPP, bornyl pyrophosphate; BPPS, BPP synthase; DMAPP, dimethylallyl pyrophosphate; FPP, farnesyl pyrophosphate; GC-MS, gas chromatography-mass spectrometry; GPP, geranyl pyrophosphate; HMG-CoA, hydroxymethylglutaryl-CoA; IPM, isopropyl myristate; IPP, isoprenyl pyrophosphate; SD, standard deviation; YPD, yeast peptone dextrose.

* Corresponding author. Engineering Biology Research Center, Kobe University, 1-1 Rokkodai, Nada, Kobe, 657-8501, Japan.

E-mail address: junjun@port.kobe-u.ac.jp (J. Ishii).

¹ Equally contributing authors.

<https://doi.org/10.1016/j.mec.2025.e00259>

Received 19 November 2024; Received in revised form 13 February 2025; Accepted 29 March 2025

Available online 31 March 2025

2214-0301/© 2025 The Authors. Published by Elsevier B.V. on behalf of International Metabolic Engineering Society. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Lippia dulcis (Despinasse et al., 2017; Wang et al., 2018; Ma et al., 2022). BDHs are found in plants (*C. camphora* [L.] Presl) (Ma et al., 2021) and bacteria (*Pseudomonas* sp. strain TCU-HL1) (Tsang et al., 2016). More recently, a plant BAT was identified for the first time in *Wurfbainia villosa* (homotypic synonym, *A. villosum*) (Liang et al., 2022). Microbial production of borneol by heterologous expression of plant BPPs has also been reported. An engineered *Escherichia coli* strain reportedly produced 87.20 mg/L borneol in shake-flask fermentation (Lei et al., 2021), whereas an engineered strain of the yeast *Saccharomyces cerevisiae* produced 12.68 mg/L and 148.59 mg/L borneol in a shake flask and 5-L bioreactor, respectively (Ma et al., 2022). However, borneol productivity remains far below the level needed for practical use, and microbial production of camphor or bornyl acetate has not been reported to date. To establish a microbial platform to produce borneol, camphor, and bornyl acetate, we metabolically engineered the yeast *S. cerevisiae*, including engineering the heterologous expression of mevalonate pathway enzymes and a fusion enzyme between BPPS and an Erg20p mutant, with and without BDH or BAT.

2. Materials and methods

2.1. Strains, plasmids, synthetic DNAs, and primers

All synthetic gene cassettes examined in the study are listed in [Supplementary Table 1](#). All synthetic DNA fragments were purchased from GeneArt (Thermo Fisher Scientific, Waltham, MA, USA). The following parental plasmids were used for plasmid construction: pRS405red (Tominaga et al., 2021) and pATP403red (Tominaga et al., 2021). Plasmid sequences are shown in [Supplementary Note 1](#). Schematic illustrations of the construction of yeast strains used in this study are shown in [Supplementary Figs. 1–3](#).

2.2. Media and reagents

Synthetic complete (SC, 0.67 % yeast nitrogen base without amino acids [BD Biosciences, San Jose, CA, USA], 2 % D-glucose [Nacalai Tesque, Kyoto, Japan], 0.2 % amino acids complete mix without L-methionine, L-leucine, L-histidine, and uracil [[Supplementary Table 2](#)]) and yeast peptone dextrose (YPD, 1 % yeast extract [Nacalai Tesque], 2 % Bacto peptone [BD Biosciences], 2 % D-glucose) media were used for incubating yeast strains. To obtain solid media, 2 % agarose was added.

2.3. Shake-flask fermentation and monoterpene quantification

An overnight culture of yeast in 5 mL of SC ([Fig. 2](#)) or YPD ([Supplementary Fig. 5](#)) medium incubated at 30 °C at 150 rpm was transferred into 20 mL of YPD medium in a baffled shake flask (initial OD₆₀₀ ~0.1) and incubated at 30 °C at 200 rpm. At each time point, the

OD₆₀₀ was monitored using a UV-1280 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan), and the cell culture was vigorously mixed with an equivalent volume of ethyl acetate by vortexing for 10 min at 4 °C, followed by centrifugation for 10 min at 13,000 g. The levels of extracted borneol, camphor, and bornyl acetate in the organic phase were measured using a gas chromatography–mass spectrometry (GC-MS) system consisting of a GCMS-QP2010 Ultra (Shimadzu) spectrometer equipped with a DB-5MS column (0.25 mm × 30 m, membrane thickness of 0.25 µm; Agilent Technologies, Santa Clara, CA, USA) and AOC-20i auto-injector (Shimadzu), as described previously (Zhang et al., 2021) with the following modifications. Mass spectrometry was carried out in the SIM/Scan mode and *m/z* range of 50–200. Calibrations were performed using standards at a concentration of up to 50 µM with a coefficient of determination >0.99. The major quantifier ion for all three analytes was *m/z* 95. Other qualifier ions were as follows: *m/z* 67 and 110 for borneol, *m/z* 81 and 108 for camphor, and *m/z* 93 and 136 for bornyl acetate. For two-phase fermentation, isopropyl myristate (IPM) was added to the medium at a final concentration of 10 % (v/v), and cultivation was carried out by vortexing for 10 min at 4 °C. Finally, a 1-mL aliquot of the organic phase was used for analysis.

3. Results and discussion

To generate a yeast strain that produces borneol, the gene encoding BPPS without the N-terminal plastid-localization sequence (2–49 residues) from *S. officinalis* (SoBPPS, GenBank: AAC26017.1) was codon-optimized for yeast ([Supplementary Table 1](#)) and fused to the mutated *ERG20* gene from yeast, which encodes mutant Erg20p with high GPP synthesis activity (Erg20p^{F96W–N127W}, Erg20p^{WW}), thereby enabling the efficient substrate channeling (Ignea et al., 2014) toward BPP. The resulting fusion gene was cloned into the yeast integration vector so that the BPPS gene could be expressed from the strong *TDH3* promoter. In addition, the genes encoding acetoacetyl-CoA thiolase/hydroxymethylglutaryl-CoA (HMG-CoA) reductase and HMG-CoA synthase from *Enterococcus faecalis* (*EfmvaE* and *EfmvaS*, respectively), which convert acetyl-CoA into mevalonate to produce more terpenoids in both *E. coli* (Tsuruta et al., 2009; Yoon et al., 2009) and the yeast *S. cerevisiae* (Peng et al., 2017), were also cloned into plasmid pATP403red (Tominaga et al., 2021). The resulting plasmids were successively integrated into the chromosome of the yeast strain SckZ014 derived from strain BY4741 (Brachmann et al., 1998), which harbors a borneol-responsive transcription activator and reporter plasmid for the *in vivo* sensing of borneol ([Supplementary Fig. 1](#) and [Supplementary Note 1](#)), thereby generating yeast strain SckZ045 ([Supplementary Fig. 2](#)). When this strain was incubated in YPD medium, the concentration of borneol in the medium increased after 24 h and peaked at 72 h, reaching 13.6 mg/L before gradually decreasing ([Fig. 2A](#) and [Supplementary Fig. 4](#)). Note that the borneol concentration was >3-fold

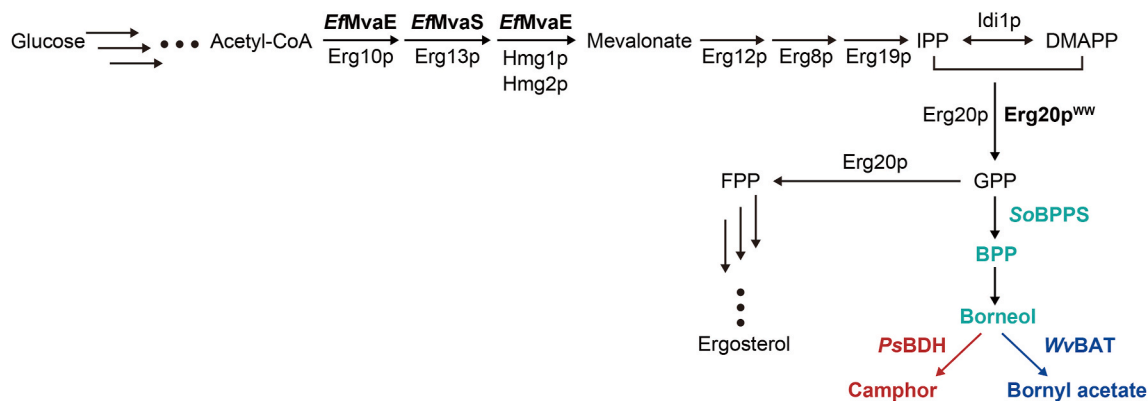


Fig. 1. Biosynthetic pathway of borneol, camphor, and bornyl acetate reconstituted in the yeast *S. cerevisiae*. Heterologous enzymes are indicated in bold type.

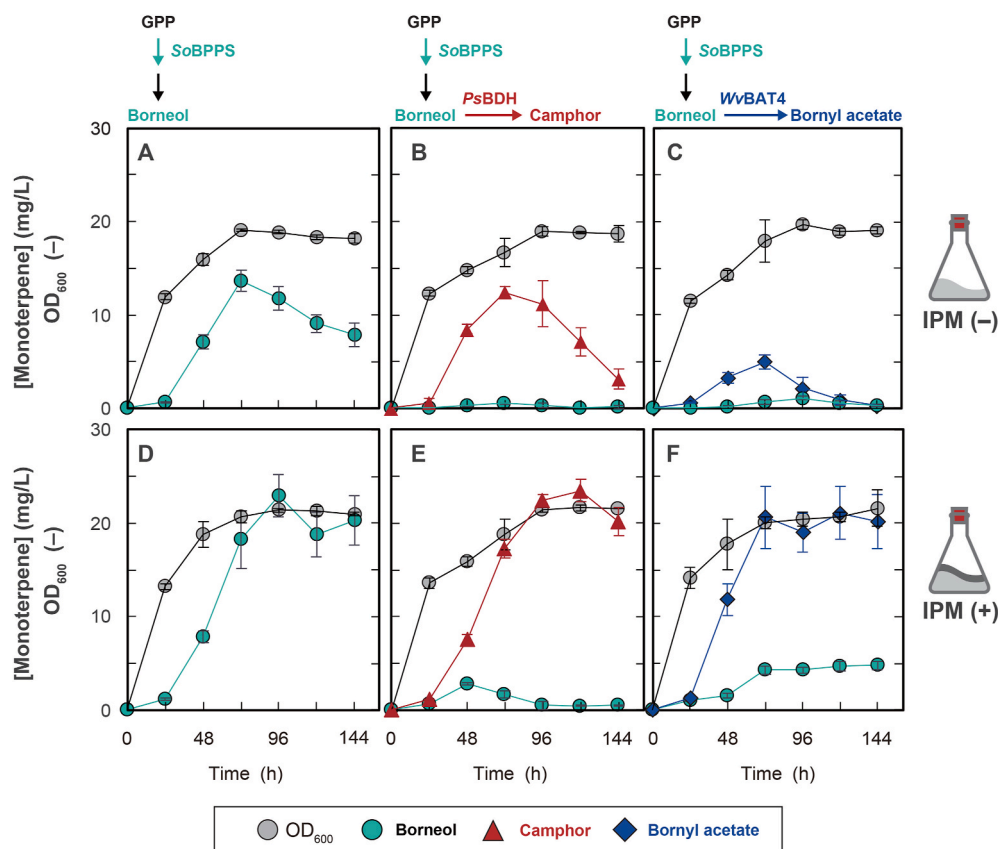


Fig. 2. Single- and two-phase shake-flask fermentation of yeasts to produce borneol, camphor, and bornyl acetate. Yeast strains expressing Erg20p^{WW}::SoBPPS, *EfMvaE*, and *EfMvaS* (ScKZ045 [A, D]) or in further combination with *PsBDH* (ScKZ048 [B, E]) or *WvBAT4* (ScKZ046 [C, F]). Yeasts were incubated in a shake flask without (A, B, C) or with (D, E, F) IPM overlay. Data are shown as the mean, and error bars show the SD of three independent experiments.

higher than that produced using the strain lacking expression of *EfMvaE* and *EfMvaS* (Supplementary Fig. 5).

To convert borneol into camphor and bornyl acetate, two strains were constructed that additionally express borneol dehydrogenase from *Pseudomonas* sp. TCU-HL1 (*PsBDH*, GenBank: AOE86728.1) and bornyl acetyltransferase from *W. villosa* (*WvBAT4*) (Liang et al., 2022). To do so, codon-optimized *PsBDH* and *WvBAT4* (Supplementary Table 1) were additionally cloned into the expression vectors for *EfMvaE* and *EfMvaS* and integrated into yeast strain ScKZ045, generating strains ScKZ048 and ScKZ046, respectively (Supplementary Fig. 3). These strains successfully produced camphor and bornyl acetate (Fig. 2B and C, and Supplementary Fig. 4) and exhibited the same time-course for borneol production. The concentrations of camphor and bornyl acetate reached 12.4 and 5.00 mg/L at 72 h, respectively, and then decreased. Borneol, camphor, and bornyl acetate are highly volatile. For instance, >20 % of borneol is lost from the medium after 48 h of incubation (Lei et al., 2021), which may contribute to the observed decrease in monoterpene production after 72 h.

To minimize the loss of volatile monoterpenes from the fermentation medium, we performed two-phase fermentation by adding IPM to the medium (Fig. 2D–F). IPM was added at a final concentration of 10 % (v/v), and cultivation was carried out by vortexing, followed by removal of an aliquot from the organic phase for analysis. As expected, the maximum concentrations of borneol, camphor, and bornyl acetate increased by 1.7-fold (23.0 mg/L at 96 h, Fig. 2D), 1.9-fold (23.5 mg/L at 120 h, Fig. 2E), and 4.2-fold (21.1 mg/L at 120 h, Fig. 2F), respectively. Note that the growth of all yeast strains was enhanced slightly by the addition of IPM, possibly because the toxic effects of the monoterpenes were reduced by extracting the compounds into the IPM phase (Brennan et al., 2012). Approximately 5 mg/L of borneol remained in the medium when producing bornyl acetate (Fig. 2F) but not when producing

camphor (Fig. 2D), indicating that the enzymatic activity of *WvBAT4* is weaker than that of *PsBDH*.

4. Conclusions

In this study, we established a microbial platform to produce borneol, camphor, and bornyl acetate using the yeast *S. cerevisiae*. To this end, we engineered yeast strains to express the rate-limiting enzymes (*EfMvaE* and *EfMvaS*) and the fusion enzyme of BPPS and Erg20p^{WW} with and without *PsBDH* or *WvBAT4*. This study is the first report of yeast production of camphor and bornyl acetate. Although the concentration of borneol produced was comparable to that of a previously reported yeast strain (Ma et al., 2022), overlaying IPM onto the medium improved borneol production by 1.7-fold, leading to the highest borneol titer in a shake flask reported for the yeast *S. cerevisiae*. Nevertheless, the borneol production was 4-fold lower than that reported for *E. coli* (Lei et al., 2021). Further improvement in production could be achieved by combining the modulation of competitive pathways (Paddon et al., 2013; Hull et al., 2014; Peng et al., 2017; Broker et al., 2018; Zhou et al., 2021; Tominaga et al., 2022; Wei et al., 2024), protein engineering to improve the enzymatic activity of BPPSs (Lei et al., 2021), amplification of the gene copy number of rate-limiting enzymes (Peng et al., 2022), and enzyme compartmentalization (Cheah et al., 2023). Evolutionary engineering for the production of borneol and camphor can be performed using recently developed genetically encoded biosensors that respond to borneol or camphor (Ikushima et al., 2015; Ikushima and Boeke, 2017; Tominaga et al., 2021; D'oelesnitz et al., 2022). Finally, a high-throughput platform could be developed to facilitate mutational analysis of various plant BDHs (Lin et al., 2023) and investigate their properties, such as product specificity (Hofer et al., 2021; Ma et al., 2021; Hu et al., 2022).

CRedit authorship contribution statement

Masahiro Tominaga: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kazuma Kawakami:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hiro Ogawa:** Methodology, Investigation, Formal analysis, Data curation. **Tomomi Nakamura:** Methodology, Formal analysis, Data curation. **Akihiko Kondo:** Supervision, Resources, Project administration, Conceptualization. **Jun Ishii:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by the GteX (grant number: JPMJGX23B4) and CREST (grant number: JPMJCR21N2) programs of the Japan Science and Technology Agency, the Intensive Support for Young Promising Researchers Program (JPNP20004) from the New Energy and Industrial Technology Development Organization (NEDO), and JSPS KAKENHI (grant numbers: JP23K26469, JP23H01776, and JP18K14374) from the Japan Society for the Promotion of Science (JSPS).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mec.2025.e00259>.

Data availability

Data will be made available on request.

References

- Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., et al., 1998. Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* 14, 115–132.
- Brennan, T.C., Turner, C.D., Kromer, J.O., Nielsen, L.K., 2012. Alleviating monoterpene toxicity using a two-phase extractive fermentation for the bioproduction of jet fuel mixtures in *Saccharomyces cerevisiae*. *Biotechnol. Bioeng.* 109, 2513–2522.
- Broker, J.N., Muller, B., Van Deenen, N., Prufer, D., Schulze Gronover, C., 2018. Upregulating the mevalonate pathway and repressing sterol synthesis in *Saccharomyces cerevisiae* enhances the production of triterpenes. *Appl. Microbiol. Biotechnol.* 102, 6923–6934.
- Cheah, L.C., Liu, L., Plan, M.R., Peng, B., Lu, Z., et al., 2023. Product profiles of promiscuous enzymes can be altered by controlling in vivo spatial organization. *Adv. Sci.* 10, e2303415.
- D'oelsnitz, S., Nguyen, V., Alper, H.S., Ellington, A.D., 2022. Evolving a generalist biosensor for bicyclic monoterpenes. *ACS Synth. Biol.* 11, 265–272.
- Despinasse, Y., Fiorucci, S., Antonczak, S., Moja, S., Bony, A., et al., 2017. Bornyl-diphosphate synthase from *Lavandula angustifolia*: a major monoterpene synthase involved in essential oil quality. *Phytochemistry* 137, 24–33.
- Hofer, M., Diener, J., Begander, B., Kourist, R., Sieber, V., 2021. Engineering of a borneol dehydrogenase from *P. putida* for the enzymatic resolution of camphor. *Appl. Microbiol. Biotechnol.* 105, 3159–3167.
- Hu, X.X., Deng, H.X., Bai, Y.J., Fan, T.P., Zheng, X.H., et al., 2022. Heterologous expression and characterization of a borneol dehydrogenase from *Arabidopsis lyrata* and its application for the enzymatic resolution of rac-camphor. *Mol. Catal.* 530, 112572.
- Hull, C.M., Loveridge, E.J., Rolley, N.J., Donnison, I.S., Kelly, S.L., et al., 2014. Co-production of ethanol and squalene using a *Saccharomyces cerevisiae* ERG1 (squalene epoxidase) mutant and agro-industrial feedstock. *Biotechnol. Biofuels* 7, 133.
- Ignéa, C., Pontini, M., Maffei, M.E., Makris, A.M., Kampranis, S.C., 2014. Engineering monoterpene production in yeast using a synthetic dominant negative geranyl diphosphate synthase. *ACS Synth. Biol.* 3, 298–306.
- Ikushima, S., Boeke, J.D., 2017. New orthogonal transcriptional switches derived from tet repressor homologues for *Saccharomyces cerevisiae* regulated by 2,4-diacetylphloroglucinol and other ligands. *ACS Synth. Biol.* 6, 497–506.
- Ikushima, S., Zhao, Y., Boeke, J.D., 2015. Development of a tightly controlled off switch for *Saccharomyces cerevisiae* regulated by camphor, a low-cost natural product. *G3 (Bethesda)* 5, 1983–1990.
- Lei, D., Qiu, Z., Wu, J., Qiao, B., Qiao, J., et al., 2021. Combining Metabolic and Monoterpene Synthase Engineering for de Novo Production of Monoterpene Alcohols in *Escherichia coli*. *ACS Synth. Biol.* 10, 1531–1544.
- Li, F.P., Huang, S.L., Mei, Y., Wu, B.Q., Hou, Z.W., et al., 2022. Genome assembly provided new insights into the evolution and d-borneol biosynthesis differences between chemotypes. *Ind. Crop. Prod.* 186, 115181.
- Liang, H., Lin, X., Yang, P., Sun, Y., Wu, Q., et al., 2022. Genome-Wide identification of BAHD superfamily and functional characterization of bornyl acetyltransferases involved in the bornyl acetate biosynthesis in *Wurfbainia villosa*. *Front. Plant Sci.* 13, 860152.
- Lin, X., Huang, L., Liang, H., Hou, C., Ling, X., et al., 2023. Genome-wide identification and functional characterization of borneol dehydrogenases in *Wurfbainia villosa*. *Planta* 258, 69.
- Ma, R., Lu, D., Wang, J., Xie, Q., Guo, J., 2023. Comparison of pharmacological activity and safety of different stereochemical configurations of borneol: L-borneol, D-borneol, and synthetic borneol. *Biomed. Pharmacother.* 164, 114668.
- Ma, R., Su, P., Jin, B., Guo, J., Tian, M., et al., 2021. Molecular cloning and functional identification of a high-efficiency (+)-borneol dehydrogenase from *Cinnamomum camphora* (L.) Presl. *Plant Physiol. Biochem.* 158, 363–371.
- Ma, R., Su, P., Ma, Q., Guo, J., Chen, S., et al., 2022. Identification of (-)-bornyl diphosphate synthase from *Blumea balsamifera* and its application for (-)-borneol biosynthesis in *Saccharomyces cerevisiae*. *Synth. Syst. Biotechnol.* 7, 490–497.
- Paddon, C.J., Westfall, P.J., Pitera, D.J., Benjamin, K., Fisher, K., et al., 2013. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nature* 496, 528–532.
- Peng, B., Esquirol, L., Lu, Z., Shen, Q., Cheah, L.C., et al., 2022. An in vivo gene amplification system for high level expression in *Saccharomyces cerevisiae*. *Nat. Commun.* 13, 2895.
- Peng, B., Plan, M.R., Chrysanthopoulos, P., Hodson, M.P., Nielsen, L.K., et al., 2017. A squalene synthase protein degradation method for improved sesquiterpene production in *Saccharomyces cerevisiae*. *Metab. Eng.* 39, 209–219.
- Ponomarev, D., Mettee, H., 2016. Camphor and its industrial synthesis. *Chem. Educ. J* 18, 1–4.
- Tominaga, M., Miyazaki, K., Hataya, S., Mitsui, Y., Kuroda, S., et al., 2022. Enhanced squalene production by modulation of pathways consuming squalene and its precursor. *J. Biosci. Bioeng.* 134, 1–6.
- Tominaga, M., Nozaki, K., Umeno, D., Ishii, J., Kondo, A., 2021. Robust and flexible platform for directed evolution of yeast genetic switches. *Nat. Commun.* 12, 1846.
- Tsang, H.L., Huang, J.L., Lin, Y.H., Huang, K.F., Lu, P.L., et al., 2016. Borneol dehydrogenase from *Pseudomonas* sp. strain TCU-HL1 catalyzes the oxidation of (+)-Borneol and its isomers to camphor. *Appl. Environ. Microbiol.* 82, 6378–6385.
- Tsuruta, H., Paddon, C.J., Eng, D., Lenihan, J.R., Horning, T., et al., 2009. High-level production of amorpho-4,11-diene, a precursor of the antimalarial agent artemisinin, in *Escherichia coli*. *PLoS One* 4, e4489.
- Wang, H., Ma, D., Yang, J., Deng, K., Li, M., et al., 2018. An integrative volatile terpenoid profiling and transcriptomics analysis for gene mining and functional characterization of AvBPPS and AvPS involved in the monoterpene biosynthesis in *Amomum villosum*. *Front. Plant Sci.* 9, 846.
- Wei, C., Zhang, L., Shen, W., Zou, W., Xia, Y., et al., 2024. Enhancement of squalene synthesis in *Candida tropicalis* via combinatorial metabolic engineering strategies to rebuild pathways. *Biochem. Eng. J.* 208, 109348.
- Yoon, S.H., Lee, S.H., Das, A., Ryu, H.K., Jang, H.J., et al., 2009. Combinatorial expression of bacterial whole mevalonate pathway for the production of beta-carotene in *E. coli*. *J. Biotechnol.* 140, 218–226.
- Zhang, C., Chen, X., Lee, R.T.C., T, R., Maurer-Stroh, S., et al., 2021. Bioinformatics-aided identification, characterization and applications of mushroom linalool synthases. *Commun. Biol.* 4, 223.
- Zhao, Z.J., Sun, Y.L., Ruan, X.F., 2023. Bornyl acetate: a promising agent in phytomedicine for inflammation and immune modulation. *Phytomedicine* 114, 154781.
- Zhou, C., Li, M., Lu, S., Cheng, Y., Guo, X., et al., 2021. Engineering of cis-element in *Saccharomyces cerevisiae* for efficient accumulation of value-added compound squalene via downregulation of the downstream metabolic flux. *J. Agric. Food Chem.* 69, 12474–12484.
- Zhou, Y., Yan, W.D., 2016. Conservation and applications of camphor tree (*Cinnamomum camphora*) in China: ethnobotany and genetic resources. *Genet. Resour. Crop Evol.* 63, 1049–1061.