Supplementary information

Producing plant volatile terpenoids (Rose Oil) by yeast cell factories

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Plasmid construction

Construction of pRS425-LEU-TEF1-*tObGES*-ERG20*

The *tObGES* was amplified from the plasmid pM3-*tObGES* (using primer sets SexAI-*tObGES*-F/*tObGES*-GGGS-ERG20-R), the ERG20*(ERG20$^{F96W-N127W}$) was amplified from the plasmid pM4-ERG20$^{F96W-N127W}$ (using primer sets *tObGES*-GGGS-ERG20-F/AscI-ERG20-R), then the *tObGES*-ERG20* was amplified with *tObGES* and ERG20* (using primer sets SexAI-*tObGES*-F/AscI-ERG20-R), and cloned into plasmid pRS425-LEU-TEF1-Pn3-29 at SexAI and AscI sites, resulting in plasmid pRS425-LEU-TEF1- *tObGES*-ERG20*.

Construction of pRS425-LEU-TEF1-ERG20*- *tObGES*

The ERG20* was amplified from the plasmid pM4-ERG20$^{F96W-N127W}$ (using primer sets SexAI-ERG20-F/ERG20-GGGS-*tObGES*-R), the *tObGES* was amplified from the plasmid pM3-*tObGES*(using primer sets ERG20-GGGS-*tObGES*-F/AscI-ERG20-R), then the ERG20*-*tObGES* was amplified with *tObGES* and ERG20* (using primer sets SexA1-ERG20-F/AscI-*tObGES*-R), and cloned into plasmid pRS425-LEU-TEF1-Pn3-29 at SexAI and AscI sites, resulting in plasmid pRS425-LEU-TEF1- ERG20*-*tObGES*.

Construction of pRS425-LEU-TEF1-CbLIS-ERG20*

The CbLIS was amplified from the plasmid pUC57-CbLIS (using primer sets SexAI-CbLIS-F/CbLIS-GGGS-ERG20-R), the ERG20* was amplified from the plasmid pM4-ERG20$^{F96W-N127W}$ (using primer sets CbLIS-GGGS-ERG20-F/AscI-ERG20-R), then the CbLIS-ERG20* was amplified with CbLIS and ERG20* (using primer sets SexAI-CbLIS-F/AscI-ERG20-R), and cloned into plasmid pRS425-LEU-TEF1-Pn3-29 at SexAI and AscI sites, resulting in plasmid pRS425-LEU-TEF1-CbLIS-ERG20*.

Construction of pRS425-LEU-TEF1-SINDPS1

Plasmid pUC57-SINDPS1 was digested with SexAI and AscI, and cloned into plasmid pRS425-LEU-TEF1-Pn3-29 at SexAI and AscI sites, resulting in plasmid pRS425-LEU-TEF1-SINDPS1.

Construction of pRS425-LEU-TRP-URA-TEF1-*tObGES*-ERG20*

The TRP1-URA3 was amplified from the plasmid pEASY-TRP-URA (using primer sets XmaI-TRP1-F/NOTI-URA3-R), and cloned into plasmid pRS425-LEU-TEF1-*tObGES*-ERG20* at XmaI and NOTI sites, resulting in plasmid pRS425-LEU-TRP-URA-TEF1-*tObGES*-ERG20*.

Construction of pM4-OYE2

The OYE2 gene was amplified from the genome DNA of *Saccharomyces cerevisiae*

(using primer sets SexAI-OYE2-F/AscI-OYE2-R), and cloned into plasmid pM4-ERG20$^{F96W-N127W}$ at SexAI and AscI sites, resulting in plasmid pM4-OYE2.

Construction of pM4-GmNES

Plasmid pUC57-GmNES was digested with SexAI and AscI, and cloned into plasmid pM4-ERG20$^{F96W-N127W}$ at SexAI and AscI sites, resulting in plasmid pM4-GmNES.

Construction of pRS425-LEU-TEF1-p425-synSmFPS-synCsVa1

The synSmFPS was amplified from the plasmid pM3-synSmFPS (using primer sets SexA1-SynSmFPS-F/SynSmFPS-GGGS-SynCsVa1-R), the synCsVa1 was amplified from the plasmid pUC57-synCsVa1 (using primer sets synSmFPS-GGGS-SynCsVa1-F/Asc1-SynCsVa1-R), then the synSmFPS-synCsVa1 was amplified with synSmFPS and synCsVa1 (using primer sets SexA1-SynSmFPS-F / Asc1-SynCsVa1-R), and cloned into plasmid pRS425-LEU-TEF1-Pn3-29 at SexAI and AscI sites, resulting in plasmid pRS425-LEU-TEF1-synSmFPS-synCsVa1.

Strain construction

Transformation of *S. cerevisiae* strains was performed as described previously (2,3).

Construction of strain HP001

Strain HP001 was constructed by integrating the *tHMG1, ERG12, IDI1, ERG19, synHMGR, ERG13, ERG8* and *ERG10* genes into the *YJL064W* site of strain CEN.PK2-1D *(MATα ura3-52; trp1-289; leu2-3,112; his3Δ1; MAL2-8C; SUC2)*. Eight DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-YJL064W-PGK1-F/ S-7G-1-M-ADHT-PDC1-R), pM9-ERG12 (using primer set S-7G-1-M-ADH1t-PDC1-F /3G-1-M-ADH2t-ENO2-R), pM11-ERG1 (using primer set 3G-3-M-ADHt-TDH3-F/ 3G-3-M-TPI1t-TEF1-R), pM11-ERG1 (using primer set 3G-3-M-ADHt-TDH3-F/ 3G-3-M-TPI1t-TEF1-R), pM16-IDI1 (using primer set 3G-2-M-ADH2t-ENO2-F/ 3G-2-M-PDC1t-PYK1-R), pM5-ERG19 (using primer set 3G-3-M-PDC1t-PYK1-F/ S-8G-1-M-PGI1t-TEF2p-R), pM7-HMGR (using primer set S-8G-1-M-PGI1t-TEF2p-F/ S-8G-1-M-ENOt-FBA1p-R),pM8-ERG13 (using primer set S-8G-1-M-ENOt-FBA1p-F/ s-4G-4-M-TDH2t–TDH3-R), pM4-ERG8 (using primer set S-4G-3M-TDH2t-TDH3-F/ 3G-3-M-TPI1t-TEF1-R) , pM3-ERG10 (using primer sets 3G-2-M-TPI1t-TEF1-F/ YJL064W-50-CYC1t-down), plasmids p414-TEF1-43802CAS9-cyc1t and YJL064W gRNA were transformed into strain CEN.PK2-1D followed by selection on SD-TRP-URA plates. All strains were verified by PCR analysis and the gRNA plasmids were removed by the SD5-FOA-TRP plates.

Construction of strains GER1, GER2

Strains GER1, GER2 were constructed by transforming the plasmids of pRS425-LEU-TEF1-*tObGES*-ERG20*and pRS425-LEU-TEF1-ERG20*-*tObGES*, respectively, into strain HP001, followed by selection on SD-TRP-LEU plates. All strains were verified by PCR analysis.

Construction of strain CIT1

Strain CIT1 was constructed by integrating the *tHMG1, tObGES-ERG20** and *OYE2* genes into the *NDT80* sites of strain HP001. Five DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-pEASY-PGK1-F/ 3G-1-M-ADHt-TDH3-R), pM4-OYE2 (using primer set 3G-3-M-ADHt-TDH3-F/ 3G-3-M-TPI1-TEF1-R), pRS425-LEU-TEF1-*tObGES*-ERG20* (using primer set 3G-2-M-TPI1-TEF1-F/ M-CYC1t-pEASY-R) and pNDT80-HIS (using primer sets X1-M-pEASY-r-t-F/ NDT80-interg.-2 and NDT80-interg.-1/ X2-M-pEASY-r-t-R) and were transformed into strain HP001 followed by selection on SD-TRP-HIS plates. All strains were verified by PCR analysis.

Construction of strains CIT1-pERG1, CIT1-pERG3, CIT1-pERG7-1, CIT1-pERG11, CIT1-pERG25, CIT1-pERG28, CIT1-pIDI1 and CIT1-pHMG1

The native ERG20 promoter was replaced by pERG1 promoter, pERG3 promoter, pERG7 promoter, pERG11 promoter, pERG25 promoter, pERG28 promoter, pIDI1 promoter, and pHMG1 promoter of strain HP001, respectively. The pERG1 promoter(807bp), pERG3 promoter(700bp), pERG7 promoter(774bp), pERG11 promoter(1000bp), pERG25 promoter(800bp), pERG28 promoter(400bp), pIDI1 promoter(1000bp) and pHMG1 promoter(1000bp) DNA fragments were amplified from genome DNA of *Saccharomyces cerevisiae* (using primer sets pERG1-F/ pERG1-R, pERG3-F/ pERG3-R, pERG7-F/ pERG7-R, pERG11-F/ pERG11-R, pERG25-F/ pERG25-R, pERG28-F/ pERG28-R, pHMG1-F/ pHMG1-R and pIDI1-F/ pIDI1-R). Then the promoter fragments was connected with the ERG20 homology arm, the pERG1-ERG20, pERG3-ERG20, pERG7-ERG20, pERG11-ERG20, pERG25-ERG20, pERG28-ERG20, pIDI1-ERG20 and pHMG1-ERG20 DNA fragments were amplified from pERG1, pERG3, pERG7, pERG11, pERG25, pERG28, pIDI1, pHMG1 DNA fragments (using primer sets pERG1-ERG20-F/ pERG1-ERG20-R, pERG3-ERG20-F/ pERG3-ERG20-R, pERG7-ERG20-F/ pERG7-ERG20-R, pERG11-ERG20-F/ pERG11-ERG20-R, pERG25-ERG20-F/ pERG25-ERG20-R, pERG28-ERG20-F/ pERG28-ERG20-R, pIDI1-ERG20-F/ pIDI1-ERG20-R, pHMG1-ERG20-F/ pHMG1-ERG20-R) . Finally, the pERG1-ERG20, pERG3-ERG20, pERG7-ERG20, pERG11-ERG20, pERG25-ERG20, pERG28-ERG20, pIDI1-ERG20, pHMG1-ERG20 DNA fragments and the pERG20gRNA were transformed into strain HP001 followed by selection on SD-TRP-URA plates, respectively. All strains were verified by PCR analysis, and the gRNA plasmids were cast away by the SD5-FOA-TRP plates.

Strains CIT-pERG1, CIT-pERG3, CIT-pERG7-1, CIT-pERG11, CIT-pERG25, CIT-pERG28, CIT-pIDI1 and CIT-pHMG1 were constructed by integrating the *tHMG1, tObGES-ERG20**and *OYE2* genes into the *NDT80* sites of strains HP001-pERG1-ERG20, HP001-pERG3-ERG20, HP001-pERG7-ERG20, HP001-pERG11-ERG20, HP001-pERG25-ERG20, HP001-pERG28-ERG20, HP001-pIDI1-ERG20 and HP001-pHMG1-ERG20. Five DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-pEASY-PGK1-F/

3G-1-M-ADHt-TDH3-R), pM4-OYE2 (using primer set 3G-3-M-ADHt-TDH3-F/ 3G-3-M-TPI1-TEF1-R) , pRS425-LEU-TEF1-*tObGES*-ERG20* (using primer set 3G-2-M-TPI1-TEF1-F/ M-CYC1t-pEASY-R) and pNDT80-HIS (using primer sets X1-M-pEASY-r-t-F/ NDT80-interg.-2 and NDT80-interg.-1/ X2-M-pEASY-r-t-R) were transformed into strains HP001-pERG1-ERG20, HP001-pERG3-ERG20, HP001-pERG7-ERG20, HP001-pERG11-ERG20, HP001-pERG25-ERG20, HP001-pERG28-ERG20, HP001-pIDI1-ERG20 and HP001-pHMG1-ERG20 followed by selection on SD-TRP-HIS plates. All strains were verified by PCR analysis.

Construction of strain CIT1-pERG7-2

Strain CIT1-pERG7-2 was constructed by transforming the plasmid of pRS425-LEU-TRP-URA-TEF1-*tObGES*-ERG20* into strain CIT1-pERG7-1 followed by selection on SD-TRP-LEU-HIS-URA plates. All strains were verified by PCR analysis.

Construction of strain HP001-pERG7-ERG9

The native ERG9 promoter was replaced by pERG7 promoter. The pERG7-ERG9 DNA fragment were amplified from pERG7 DNA fragment (using primer sets pERG7-ERG9-F/pERG7-ERG9-R) and pERG9gRNA were transformed into strain HP001 followed by selection on SD-TRP-URA plates. All strains were verified by PCR analysis.

Construction of strains NEROL and NEROL-pERG7

Strains NEROL and NEROL-pERG7 were constructed by integrating the *tHMG1, SINDPS1* and *GmNES* genes into the *NDT80* sites of strains HP001 and HP001-pERG7-ERG20. Five DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-pEASY-PGK1-F/ 3G-1-M-ADHt-TDH3-R), pM4-GmNES (using primer set 3G-2-M-ADHt-TDH3-F/ 3G-3-M-TPI1-TEF1-R), pRS425-LEU-TEF1-SINDPS1 (using primer set 3G-3-M-TPI1-TEF1-F/ M-CYC1t-pEASY-R) and pNDT80-HIS (using primer sets X1-M-pEASY-r-t-F/ NDT80-interg.-2 and NDT80-interg.-1/ X2-M-pEASY-r-t-R) and were transformed into strains HP001 and HP001-pERG7-ERG20 followed by selection on SD-TRP-HIS plates. All strains were verified by PCR analysis.

Construction of strains Rose–yeast

Strains Rose–yeast was constructed by integrating the *tHMG1, tObGES-ERG20** and *OYE2* genes into the *GAL7* sites of strain Nerol-pERG7. Five DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-pEASY-PGK1-F/3G-1-M-ADHt-TDH3-R), pM4-OYE2 (using primer set 3G-3-M-ADHt-TDH3-F/3G-3-M-TPI1-TEF1-R) , pRS425-LEU-TEF1-*tObGES*-ERG20* (using primer set 3G-2-M-TPI1-TEF1-F/M-CYC1t-R) and pGAL7-URA3 (using primer sets X1-M-pEASY-r-t-F/ GAL7-URA3-2 and GAL7-URA3-1/X2-M-pEASY-r-t-R) were transformed into strain NEROL-pERG7 followed by selection on SD-TRP-HIS-URA plates. All strains were verified by PCR analysis.

Construction of strains Rose–yeast 1.0

Strain Rose–yeast 1.0 was constructed by transforming the plasmids of pRS425-LEU-TRP-URA-TEF1-*tObGES*-ERG20* into strain Rose–yeast followed by selection on

SD-TRP-LEU-HIS-URA plates. All strains were verified by PCR analysis.

Construction of strains LIN and LIN-pERG7

Strains LIN and pERG7-LIN were constructed by integrating the *tHMG1, CBSLIS* genes into the *NDT80* sites of strain HP001 and HP001-pERG7-ERG20. Four DNA fragments were amplified from pM2-tHMG1 (using primer set 1-M-pEASY-PGK1-F/ 2G-1-M-ADHt-TEF1-R), pRS425-LEU-TEF1-CbLIS-ERG20* (using primer set 2G-2-M-ADHt-TEF1-F/ M-CYC1t-pEASY-R) and pNDT80-HIS (using primer sets X1-M-pEASY-r-t-F/ NDT80-interg.-2 and NDT80-interg.-1/ X2-M-pEASY-r-t-R) were transformed into strain HP001 and HP001-pERG7-ERG20 followed by selection on SD-TRP-HIS plates. All strains were verified by PCR analysis.

Construction of strains NED and NED-pERG7

Strains NED and NED-pERG7 were constructed by transforming the plasmid of pRS313-LEU-TEF1-FPS-optiNES into strains HP001 and HP001-pERG7-ERG9 respect -ively, followed by selection on SD-TRP-LEU plates. All strains were verified by PCR analysis.

Construction of strains VAL and VAL-pERG7

Strains VAL and VAL-pERG7 were constructed by transforming the plasmid of pRS425-LEU-TEF1-synSmFPS-synCsVa1 into strains HP001 and HP001-pERG7-ERG9, respectively, followed by selection on SD-TRP-LEU plates. All strains were verified by PCR analysis.

Chemical analysis.

A single colony of the transformants were picked up from SD agar plates and inoculated into 10 ml tubes containing 3 ml SD medium and cultivated at 30 °C, 250 rpm for 20–24h to the exponential phase. Then the seed culture was transferred into another 100 ml flasks containing 15 ml fresh SD medium with an initial OD of 0.2 and incubated at 30 °C, 250 rpm for 120h before harvest. According to Brennan et al. (2012), 10% (v/v) Methyl oleate (purchased from Sigma-Aldrich, China) or 10% (v/v) Dodecane (purchased from Sigma-Aldrich, China) were added to the culture at the beginning of the fermentation to enrich the monoterpenoids productions or sesquiterpenes productions, respectively, which could minimize the loss of the products and protect the cells from phase the toxicity brought by products.

GC-MS analysis for monoterpenoids: For quantification of monoterpenoids productions, the two-phase subcultures were centrifuged at 6000 rpm at 20 °C for 5 min to separate the Methyl oleate and aqueous layers. The 100μL Methyl oleate layer was added to the tube with an organic phase consisting of 900μL N-hexane. The organic layers were analysed on an Agilent Technologies GC-MS7890B/5973 equipped with a HP-5ms (30 m×0.25mm×0.5 um) column. Compound separation was achieved with an injector temperature of 250 °C and a 31 min temperature gradient program for GC-

separation starting at 45 °C for 1 min followed by heating the column to 130 °C at 5°C min^{-1} and then ramped at 10 °C min^{-1} to 250 °C, a final constant hold at 250°C for 1 min. Mass detection was achieved with electric ionisation using Full Scan mode with diagnostic ions monitored as followed: 50-750 amu. Geraniol (purchased from aladding, China), Citronellol (purchased from shyuanye, China), Linalool (purchased from shyuanye, China) and Nerol (purchased from shyuanye, China) samples were used as the standard for analysis.

GC-MS analysis for strains Rose-yeast 1.0 productions: For quantification of nerol and citronellol, the two-phase subcultures were centrifuged at 6000 rpm at 20 °C for 5 min to separate the Methyl oleate and aqueous layers. The 100μL Methyl oleate layer was added to the tube with an organic phase consisting of 900μL N-hexane. The organic layers were analyzed on an Agilent Technologies GC-MS7890B/5973 equipped with a HP-5ms (30 m×0.25mm×0.5 um) column. Compound separation was achieved with an injector temperature of 250 °C and a 79 min temperature gradient program for GC-separation starting at 70 °C for 35 min followed by heating the column to 115 °C at 3°C min^{-1} and then ramped at 10 °C min^{-1} to 300 °C, a final constant hold at 300°C for 10 min. Mass detection was achieved with electric ionisation using Full Scan mode with diagnostic ions monitored as followed: 50-750 amu. Geraniol (purchased from aladding, China), Citronellol (purchased from shyuanye, China), and Nerol (purchased from shyuanye, China) samples were used as the standard for analysis.

GC-MS analysis for sesquiterpenes productions: For quantification of sesquiterpenes productions, the two-phase subcultures were centrifuged at 6000 rpm at 20 °C for 5 min to separate the Dodecane and aqueous layers. The 100μL Dodecane layer was added to the tube with an organic phase consisting of 900μL N-hexane. The organic layers were analyzed on an Agilent Technologies GC-MS7890B/5973 equipped with a HP-5ms (30 m×0.25mm×0.5 um) column. Compound separation was achieved with an injector temperature of 250 °C and a 31.5 min temperature gradient program for GC-separation starting at 45 °C for 1 min followed by heating the column to 300 °C at 10°C min^{-1} and a final constant hold at 300°C for 5 min. Mass detection was achieved with electric ionisation using Full Scan mode with diagnostic ions monitored as followed: 50-750 amu. Nerolidol (purchased from shyuanye, China) and Valencene (purchased from shyuanye, China) samples were used as the standard for analysis.

Compared with current extraction from plant sources, the synthetic biology platform strategy possesses several advantages

Using engineering yeast to produce high-value plant natural products(PNPs) can solve many weaknesses in plant cultivation. The rapid generation times and high cell densities achieved in microbial fermentations enable production of target compounds

with reduced time, space and resource requirements relative to plant extraction. Cultivation in closed bioreactors can also reduce supply chain susceptibility to environmental and geopolitical disruption, while providing improved batch-to-batch consistency and active ingredient purity[7]. In summary, compared with current extraction from plant sources, the synthetic biology platform strategy possesses several advantages, such as short-cycle manufacturing, land savings, and controllable culture conditions.

Estimation of reduction in land use for transitioning plant-based rose essential oil production to yeast-based fermentation.

According to the calculation method by P. Srinivasan and C. D. Smolke (Nature, 2020)[7], we estimated the reduction in land use to prove the bioprocess with a more economical and ecological character than using the conventional process.

- Calculation of space requirements for rose flowers essential oil extraction:

The rose essential oil content in rose is ~0.017 -0.043%[8]. According to the latest report, the western Himalaya damask rose (Rosa damascena Mill.) havs mean annual productivities of 1.6 kg of rose essential oil per ha, this is quite high for rose flowers[9].

Unit conversion: 1.6 kg essential oil / ha = 1.6 kg rose essential oil / 10,000 m^2 = 1.6 g rose essential oil / 10 m^2 = 0.16 g rose essential oil / m^2

- Calculation of space requirements for yeast-based rose essential oil production platform:

Sugarcane land efficiency is ~60 tons plant biomass / ha, with a yield of 135 kg sucrose / ton plant biomass→ therefore, ~8000 kg sugar / ha = 800 g sugar / m^2

In our study, the fermentation produced 24.35g yeast-based rose essential oil and consumed about 4000 g sugar:

24.35g yeast-based rose essential oil / (4000 g sugar / 800 g sugar / m^2) = 4.87g yeast-based rose essential oil / m^2

Therefore, the reduction in required land area for yeast-based rose essential oil is: (4.87g yeast-based rose essential oil / m^2) / (0.16 g rose essential oil / m^2)= ~30-fold.

Comparison of time use for transitioning plant-based essential oil production to yeast-based fermentation.

Damask rose flowers annually once in hilly areas and its flowering period lasts for about 35–45 days (from the first week of April to second half of May) in northern India8. The fermentation to product yeast-based rose essential oil usually takes about 8-10 days, and it is little affected by climate or environmental factors. It is obviously that the production of rose essential oil by yeast cell factory has the great advantage in time saving.

Supplementary Methods Reference

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Supplementary Figure

Fig. S1 Identification of fermentation products of strain GER1. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Geraniol standard, and the Methyl oleate phase of strains GER1 and HP001; (B) Mass spectra of Geraniol.

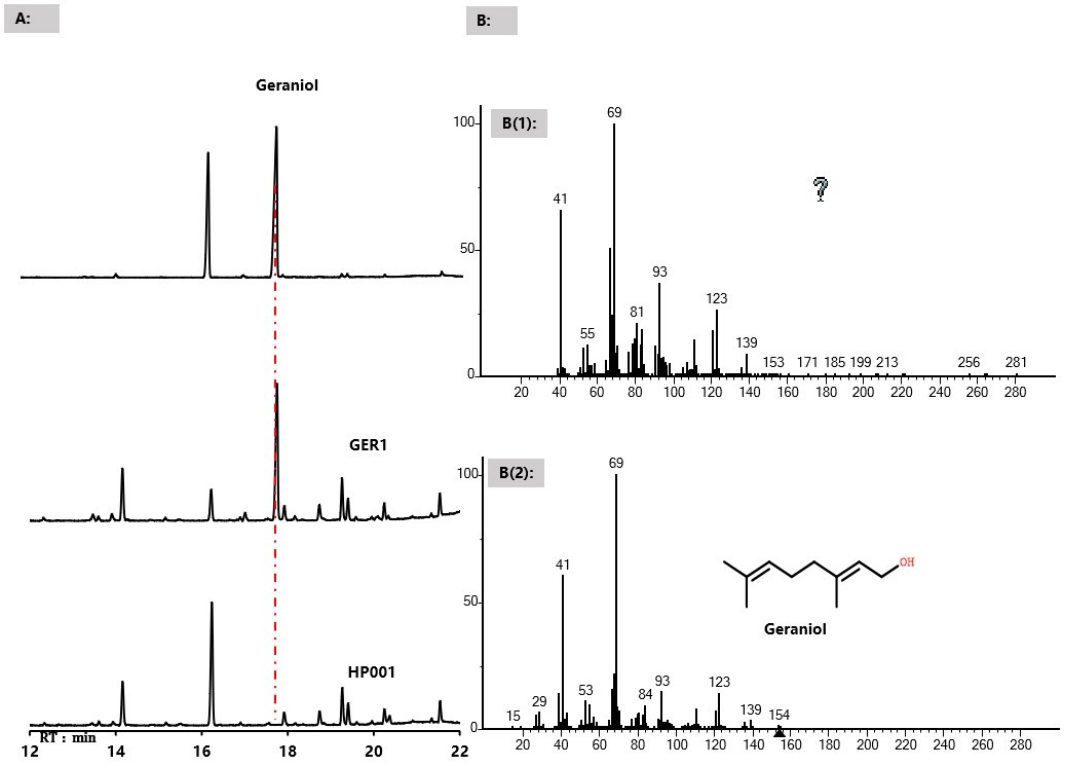


Fig.S2 Identification of fermentation products of strain CIT1. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Geraniol standard, and the Methyl oleate phase of strains CIT1 and HP001; (B) Mass spectra of Geraniol and Citronellol.

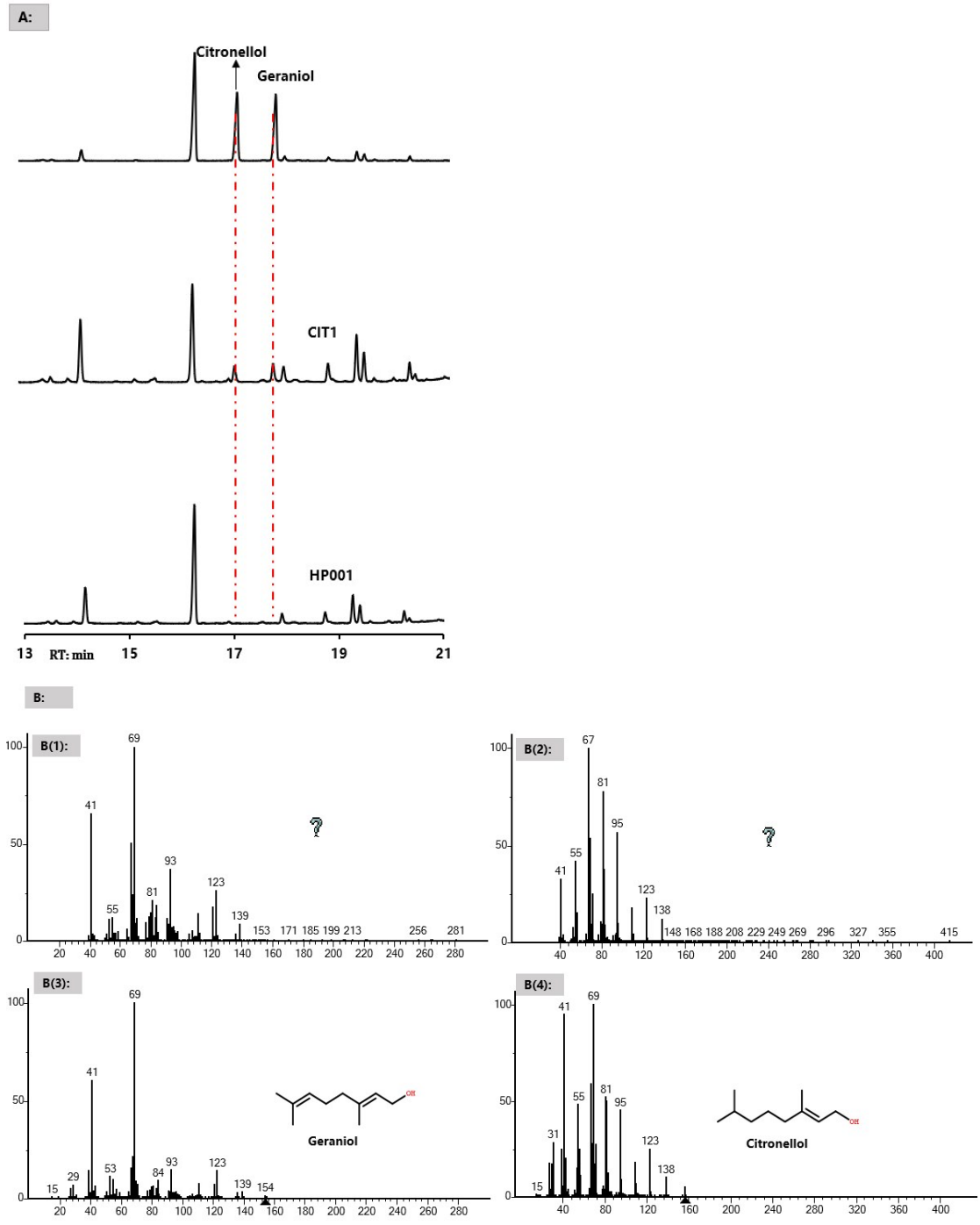


Fig. S3 Identification of the other components in the solution of samples from strain CIT1, GER1 and HP001.

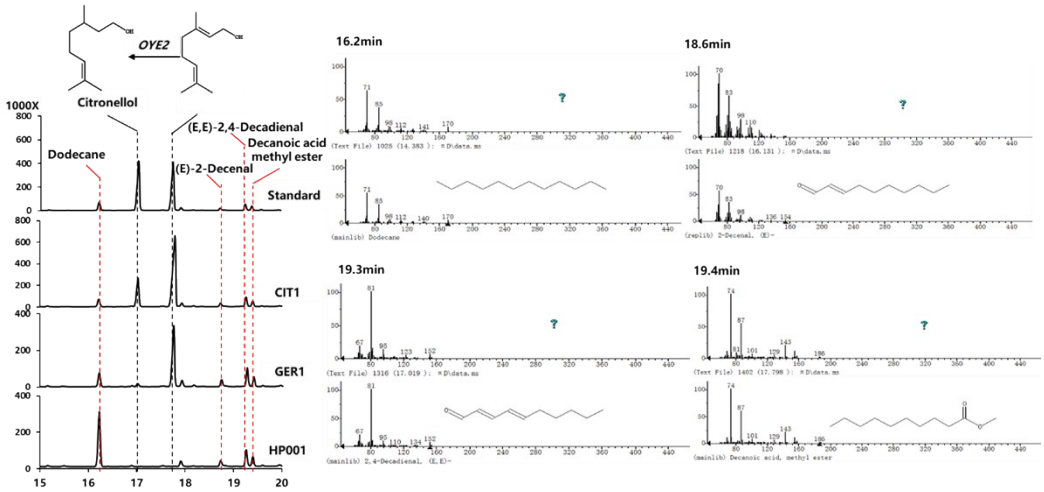


Fig. S4 Identification of fermentation products of strain LIN. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Linalool standard, and the Methyl oleate phase of strains LIN and HP001; (B) Mass spectra of Linalool.

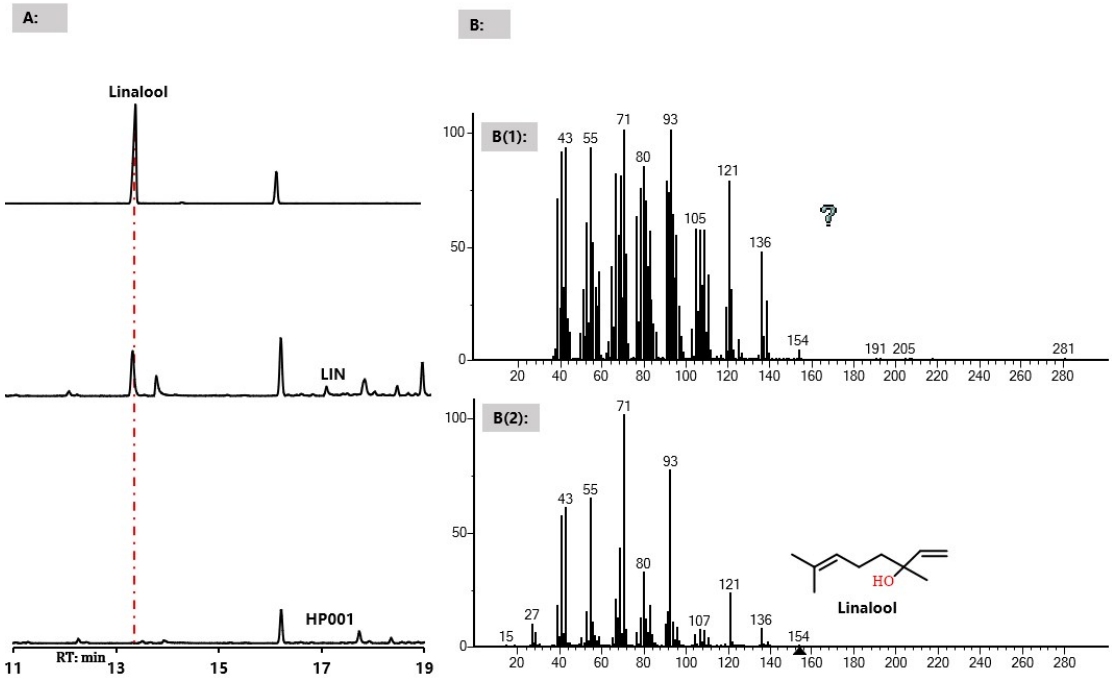


Fig. S5 Identification of fermentation products of strain NEROL. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Nerol standard, and the Methyl oleate phase of strains NEROL and HP001; (B) Mass spectra of Nerol.

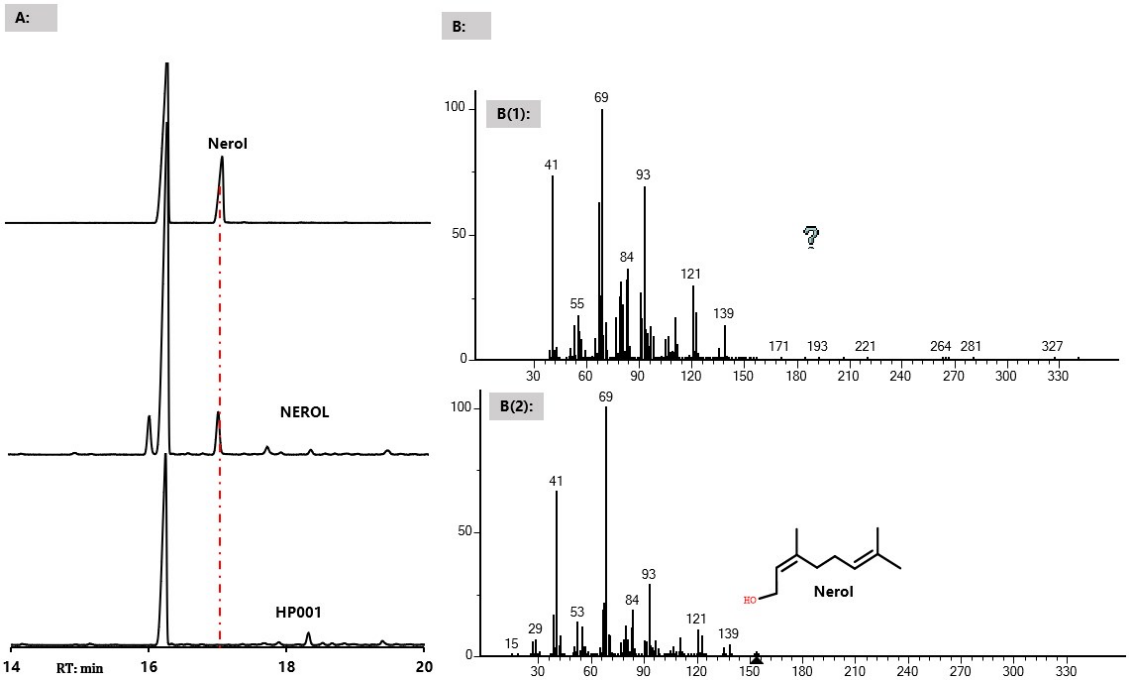


Fig. S6 Identification of fermentation products of strain VAL. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Linalool standard, and the Dodecane phase of strains VAL and HP001; (B) Mass spectra of Valencene.

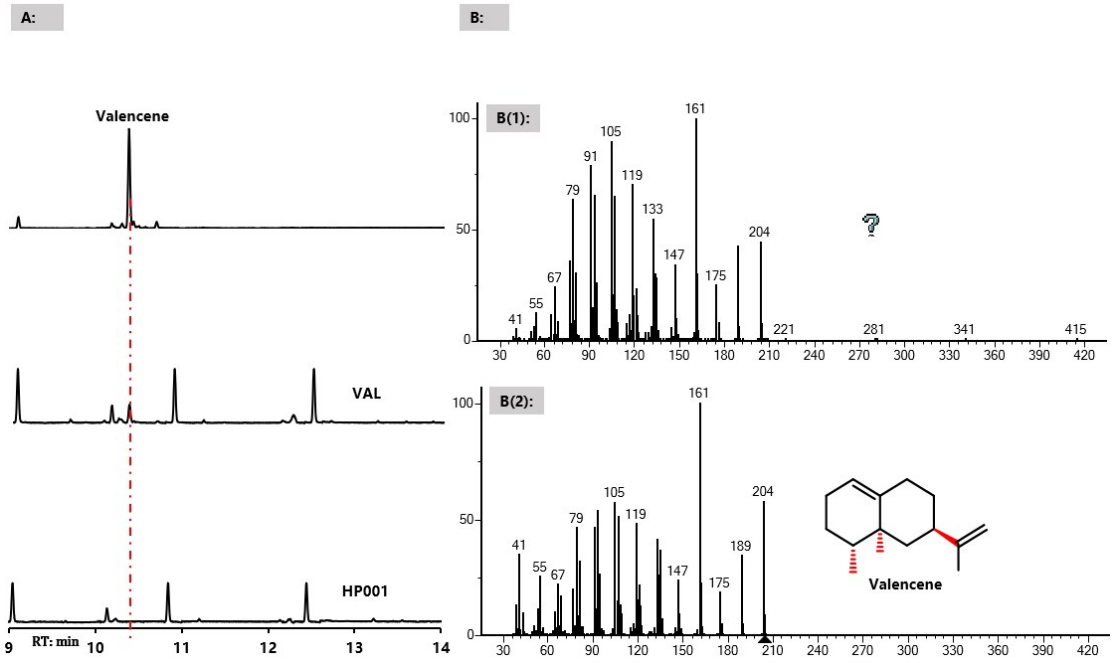
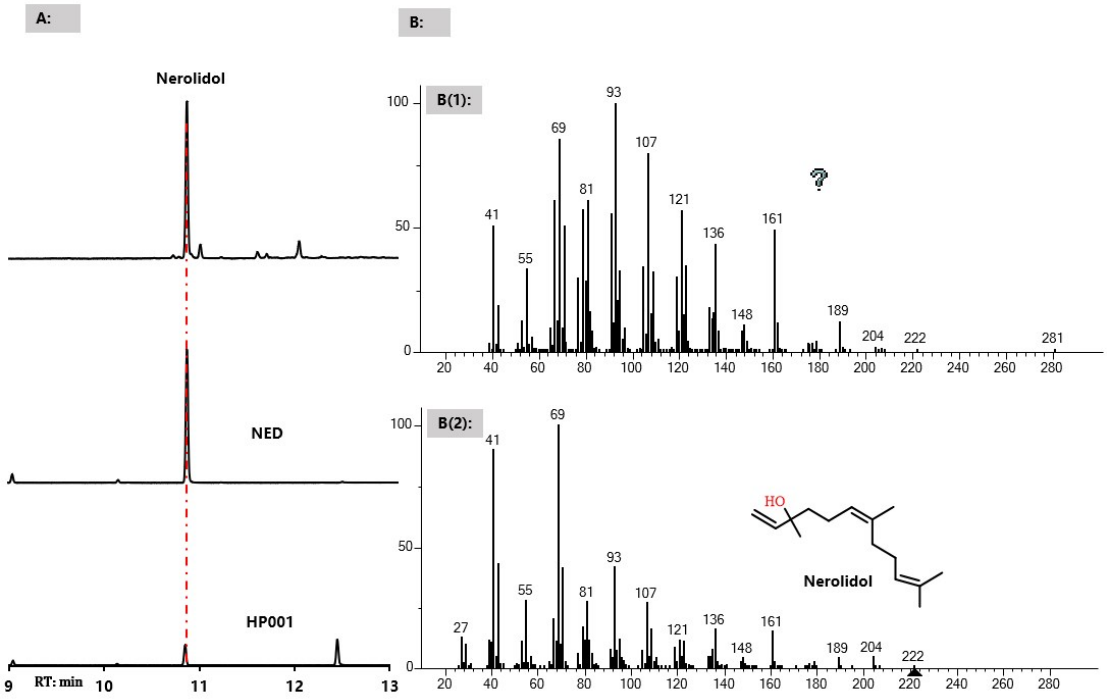


Fig. S7 Identification of fermentation products of strain NED. This strain was cultivated in SD medium with 20g/L glucose for 5 days. (A) GC-MS analysis of Nerolidol standard, and the Dodecane phase of strains NED and HP001; (B) Mass spectra of Nerolidol.



Supplementary Tables

Table S1. Primers used in this work

Primer	Sequence
SexAI-*tObGES*-F	**GCGACCTGGT**ATGTCTGCTTGTACTCCTTTGGC
ASCI-*tObGES*-R	**GCGGCGCGCC**TTATTGAGTGAAAAATAAAGCATCAACGTAATTGTC
ERG20*-GGGS-*tObGES*-F	GGTGGTGGTTCTATGTCTGCTTGTACTCCTTTGGC
tObGES-GGGS-ERG20*-F	GGTGGTGGTTCTATGGCTTCAGAAAAAGAAATTAGGAGAGAG
tObGES-GGGS-ERG20*-R	GAAGCCATAGAACCACCACCTTGAGTGAAAAATAAAGCATCAACGTAATTGTC
SexAI-OYE2-UP-F	**GCGACCTGGT**ATGCCATTTGTTAAGGACTTTAAGCC
ASCI-OYE2-DOWN-R	**GCGGCGCGCC**TTAATTTTTGTCCCAACCGAGTTTTAGAGC
tObGES-F-CE	GTCGCTTTGAACATGGCTAGG
SexAI-CbLIS-UP-F	**GCGACCTGGT**ATGCAATTGATCACTAACTTTTCTTCATCTTCAT
ASCI-CbLIS-DOWN-R	**GCGGCGCGCC**TTATGAAAAACACAATTTGATGTTTGGACCTG
ERG20*-GGGS-CbLIS-F	GAAGCAAAGGTGGTGGTTCTATGCAATTGATCACTAACTTTTCTTCATCTTC
ERG20*-GGGS-CbLIS-R	AATTGCATAGAACCACCACCTTTGCTTCTCTTGTAAACTTTGTTCAAGAACG
CbLIS-GGGS- ERG20*-F	GTTTTTCAGGTGGTGGTTCTATGGCTTCAGAAAAAGAAATTAGGAGAGAG
CbLIS-GGGS- ERG20*-R	GAAGCCATAGAACCACCACCTGAAAAACACAATTTGATGTTTGGACCTG
ASCI-SINPPS1-R	TTAATATGTATGACCACCGAATCTTCTATGTCTTTG
ASCI-OYE2-R	TTAATTTTTGTCCCAACCGAGTTTTAGAGC

ASCI-GmNES-R	TTATTCAATAACAAATTGTAAACACAAAATATGAACAACATG
pHMG1-F	CGGCAAAAAAAGCGTTATCTTCTTTC
pHMG1-R	GCTTGTTTTATGTATTTATCTACTTTGTATCAACAATTAGAC
pIDI1-F	GCTTTTGCAGATTCCGGTATTTGTG
pIDI1-R	TGAGGTGTAATGAATTTTTGGCTTATTTTTACTC
pERG3-F	GGCACCACTACGCTATAATATATGATGC
pERG3-R	ATCTCAAATCTAGACGAATATTTTTCTTATTATCTTTTTTTAC
pERG11-F	CTTGTTCTCTCTCGCTTCCTACG
pERG11-R	CCTTGTATTACTCGTTTGTTCTGTTTCTATTC
pERG25-F	ATGTAAAAGTAGATTCGCATCTGAGAATGT
pERG25-R	CTTTTCCTCTTTTTTTTATGGCTGTACTAC
pERG28-F	TCGAAAGCTCTTTCATTTCGGGG
pERG28-R	TATCTGATACTTTAGTGTATTTGAATCCTTCGATAC
pERG1-F	AGTCTTGTCGAATACTACTATGACCGC
pERG1-R	GACCCTTTTCTCGATATGTTTTTCTGTG
pERG7-F	ATGGTGCACAGTTGGTTTGTTTTAAC
pERG7-R	CTGTTTTGTACTTTCTTTGTGGGCG
pHMG1-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGCGGCAAAAAAAGCGTTATCTTCTTTC
pHMG1-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATGCTTGTTTTATGTATTTATCTACTTTGTATC AACAATT
pIDI1-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGGCTTTTGCAGATTCCGGTATTTGTG
pIDI1-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATTGAGGTGTAATGAATTTTTGGCTTATTTTT ACTC

pERG3-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGGGCACCACTACGCTATAATATATGATGC
pERG3-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATATCTCAAATCTAGACGAATATTTTTCTTATTATCTTTT
pERG11-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGCTTGTTCTCTCTCGCTTCCTACG
pERG11-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATCCTTGTATTACTCGTTTGTTCTGTTTCTATTC
pERG25-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGATGTAAAAGTAGATTCGCATCTGAGAATGT
pERG25-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATCTTTTCCTCTTTTTTTTATGGCTGTACTAC
pERG28-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGTCGAAAGCTCTTTCATTTCGGGG
pERG28-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATTATCTGATACTTTAGTGTATTTGAATCCTTCGATAC
pERG1-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGAGTCTTGTCGAATACTACTATGACCGC
pERG1-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATGACCCTTTTCTCGATATGTTTTTCTGTG
pERG7-ERG20-F	GTATATTCACCGTCCTCTACACATATTTTAAATACTGATGAAGTGACAAGATGGTGCACAGTTGGTTTGTTTTAAC
pERG7-ERG20-R	GGGAAAACGTTCAAGAATCTCTCTCTCCTAATTTCTTTTTCTGAAGCCATCTGTTTTGTACTTTCTTTGTGGGCG
ERG20-OUT-F	TGGTTGACGCCATCTGAGAATTC
ERG20-R	CTATTTGCTTCTCTTGTAAACTTTGTTCAAGAAC
TEF1-F	AGTGATCCCCCACACACCATAGCTTCAAAATGTTTCTA
TDH3-F	AAATACTAGCGTTGAATGTTAGCGTCA
PGK1-F	ACGCACAGATATTATAACATC
pERG20-N20	GCAGTGAAAGATAAATGATCCGAAGTCAGCTTCTTCTCGTGTTTTAGAGCTAGAAATAGC
pERG9-N20	GCAGTGAAAGATAAATGATCCCACTGCACTTTGCATCGGAGTTTTAGAGCTAGAAATAGC

gRNA-R	GATCATTTATCTTTCACTGC
ERG20-DOWN-R	CTATTTGCTTCTCTTGTAAACTTTGTTCAAGAAC
ERG9-DOWN-R	TCACGCTCTGTGTAAAGTGTATATATAATAAAACC
SexAI-SynSmFPS-F	GCG**ACCTGGT**ATGGCTAATTTGAATGGTGAATCTGC
SynSmFPS-GGGS-SynCsVa1-R	AGAACCACCACCTTTTTGTCTTTTATAGATTTTACCCAAAAATGATTTCAAAAC
SynSmFPS-GGGS-SynCsVa1-F	AGACAAAAAGGTGGTGGTTCTATGTCTTCAGGTGAAACTTTTAGACCAAC
AscI-SynCsVa1-R	GC**GGCGCGCC**TTAAAATGGAACATGGTCACCCAAAAC
AscI-optiNES-R	GC**GGCGCGCC**TTATAAAGAAGTATTATAGATCATT
XmaI-TRP1-F	GG**CCCGGG**TACAATCTTGATCCGGAGCTTTTC
NOTI-URA-R	CGC**GCGGCCGC**ACTCTTCCTTTTTCAATGGGTAATAAC

[2] Nucleotides indicating restriction sites were underlined and bold.

Table S2. Primers used in DNA assembly

Primer	Sequence
YJL064W-50-PGK1-F	CATCATTCAAAAACTGCAGATGGCCGTCAGAAGAGTGCACAATTGCGGCAACGCACAGATATTATAACATC
YJL064W-50-CYC1-R	ACAGAAGAAACAAGAGAGAATAGCGTCAGGATAGCTCGCTCGATGTGATCGCGCGTTGGCCGATTCATTAATG
S-7G-1-M-ADHT-PDC1-R	CCTTGCTTGTTTATCTTGCACATCACATCAGCGGAACATATGCTCACCCAGTCGCATGTCGGCATGCCGGTAGAGGTGT GGTCAATAAG
S-7G-1-M-ADH1t-PDC1-F	GGTCAGGTTGCTTTCTCAGGTATAGCATGAGGTCGCTCTTATTGACCACACCTCTACCGGCATGCCGACATGCGACTG GGTGAGCATATGTTCCG
3G-1-M-ADH2t-ENO2-R	TGTTAATCCACCTTCTAGCTCATTTTGGATGGCAACGGCAAGAGTAGGATTTAGAATTATATAACTTGATGAGATGAG
3G-2-M-ADH2t-ENO2-F	GGTAATTCTTCTGTCATTTACTCATCTCATCTCATCAAGTTATATAATTCTAAATCCTACTCTTGCCGTTGCCATCC
3G-2-M-PDC1t-PYK1-R	CCTCTTTTTAATATTGTTTTGTACTGAGATTAATCTCCAAAATAGTAGCATTGGCAGTTTTGAATTGAGTAACCATTAT
3G-3-M-PDC1t-PYK1-F	TAGAAGACTAGACACCTCGATACAAATAATGGTTACTCAATTCAAAACTGCCAATGCTACTATTTTGGAGATTAATCTC
S-8G-1-M-PGI1t-TEF2p-R	CAGCCGGCAGGGGAAGCGCCTACGCTTGACATCTACTATATGTAAGTATACGGCCCCA GGTATACTGGAGGCTTCATGAGT
S-8G-1-M-PGI1t-TEF2p-F	AGTTTCAAAGATGAATCAGTGCGCGAAGGACATAACTCATGAAGCCTCCAGTATACCTGGGGCCGTATACTTACATATA G
S-8G-1-M-ENOt-FBA1p-R	GTTATTTACAGAAGTTGGAAGGCTGGTATTGTTGTTCAAGCCAGCGGTGCCAGTTGGATCAGGTATCATCTCCATCTCC CATA
S-8G-1-M-ENOt-FBA1p-F	GGAAATGCGGGCCACGACCACAGTGATATGCATATGGGAGATGGAGATGATACCTGATCCAACTGGCACCGCTGGCTT G
s-4G-4-M-TDH2t–TDH3-R	GGCCTCCGCGTCATTAAACTTCTTGTTGTTGACGCTAACATTCAACGCTAGTATGGCGAAAAGCCAATTAGTGTGATAC
S-4G-3M-TDH2t-TDH3-F	GGCATCACGGATTTTCGATAAAGCACTTAGTATCACACTAATTGGCTTTTCGCCATACTAGCGTTGAATGTTAGCGTCAA

	C
X1-M-pEASY-r-t-F	CTTGCAAATGCCTATTGTGCAGATGTTATAATATCTGTGCGTTTAATTAAGGCTCGTATGTTGTGTGGAATTGT
X2-M-pEASY-r-t-R	CGAAGGCTTTAATTTGCAAGCTGCGGCCCTGCATTAATGAATCGGCCAACGCGCCAGGGTTTTCCCAGTCACGACGTT G
1-M-pEASY-PGK1-F	CTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCTTAATTAAACGCACAGATATTATAAC
3G-1-M-ADHt-TDH3-R	CCTCCGCGTCATTAAACTTCTTGTTGTTGACGCTAACATCAACGCTAGTATTCGGCATGCCGGTAGAGGTGTGG
3G-3-M-ADHt-TDH3-F	CAGGTATAGCATGAGGTCGCTCTTATTGACCACACCTCTACCGGCATGCCGAATACTAGCGTTGAATGTTAGCGTC
3G-3-M-TPI1t-TEF1-R	AGGAGTAGAAACATTTTGAAGCTATGGTGTGTGGGGGATCACTTTAATTAATCTATATAACAGTTGAAATTTGGA
3G-2-M-TPI1t-TEF1-F	GTCATTTTCGCGTTGAGAAGATGTTCTTATCCAAATTTCAACTGTTATATAGATTAATTAAAGTGATCCCCCACAC
M-CYC1t-pEASY-R	CGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGCGTTGGCCGA TTCA TTAATGC
1-M-ADHt-TEF1-R	GGAGTAGAAACATTTTGAAGCTATGGTGTGTGGGGGATCACTTTAATTAATCGGCA TGCCGGTAGAGGTG
2-M-ADHt-TEF1-F	GGTATAGCATGAGGTCGCTCTTATTGACCACACCTCTACCGGCATGCCGATTAATTAAAGTGATCCCCCA
X1-Xp-pEASY-M13R-F	CCGACTGGAAAGCGGGCAGTGAGCG
X2-Xp-pEASY-M13F-R	CCATTCAGGCTGCGCAACTGTTGGGA
Xp-M-pEASY-M13R-F	GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGTCACACAGGAAACAGC TATGACC
Xp-M-pEASY-M13F-R	CTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGACGACGTTGTAAAAC GACGGCCAGT
GAL7-1	ATATTTGAAAGGCTTATGATTTTCTCTTGC
GAL7-2	GTCCAAATATTTGAAAACAAAGGTACAGC
NDT80-interg.-1	CATCATAAGGAATTCCGGGATTCTCCCCAT
NDT80-interg.-2	CTGGCTTTAAAAAATGGATAAAAAGGGATG

Table S3. The GC-MS analysis of fermentation products of strain Rose–yeast 1.0. and Damask Rose Oil (purchased from JD.COM ,China).

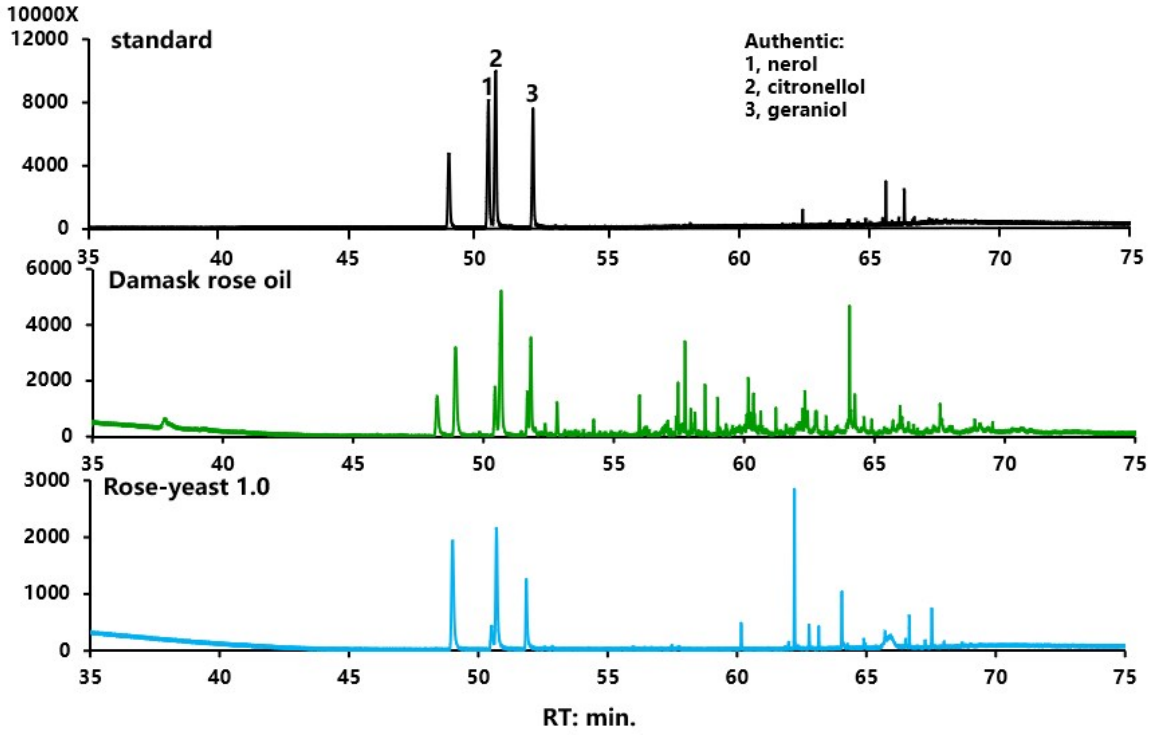


Table S3. 1 the GC-MS analysis of Damask Rose Oil.

NO	RT.	Name	Formula	Prob(%)
1	37.7	Linalool	$C_{10}H_{18}O$	94.9
2	48.2	α-Terpineol	$C_{10}H_{18}O$	64.1
3	48.9	Dodecane	$C_{12}H_{26}$	8.69
4	50.4	Nerol	$C_{10}H_{18}O$	63.7
5	50.6	Citronellol	$C_{10}H_{18}O$	12.0
6	51.6	Benzene, 1,3-bis(1,1-dimethylethyl)-	$C_{14}H_{22}$	75.3
7	51.8	Geraniol	$C_{10}H_{18}O$	41.9
8	51.9	Tetradecane	$C_{14}H_{30}$	7.9
9	52.3	Dodecane, 2,6,10-trimethyl-	$C_{15}H_{32}$	4.54
10	52.8	Dodecane, 2,7,10-trimethyl-	$C_{15}H_{32}$	7.28
11	54.2	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	7.67
12	55.9	Tetradecane	$C_{14}H_{30}$	12.3
13	56.1	Hexadecane	$C_{16}H_{34}$	9.56
14	56.2	Nonadecane	$C_{19}H_{40}$	6.36
15	56.8	Heneicosane	$C_{21}H_{44}$	6.59
16	56.9	Hexadecane	$C_{16}H_{34}$	6.39
17	57.0	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	5.66
18	57.3	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	4.91
19	57.4	Heptacosane	$C_{27}H_{56}$	5.08
20	57.5	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	4.70
21	57.6	Nonadecane	$C_{19}H_{40}$	13.7
22	57.7	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	
23	57.9	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	62.2
24	58.1	Octadecane, 2-methyl-	$C_{19}H_{40}$	6.61
25	58.5	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)-	$C_{15}H_{26}O$	57.9
26	58.9	Heptadecane	$C_{16}H_{34}$	15.6
27	59.0	Octadecane	$C_{18}H_{38}$	12.1
28	59.3	Heptacosane	$C_{27}H_{56}$	7.84
29	59.5	Eicosane	$C_{20}H_{42}$	5.42
30	59.7	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	4.21
31	59.9	Eicosane, 10-methyl-	$C_{21}H_{44}$	1.60
32	60.0	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	6.15
33	60.1	Heneicosane	$C_{21}H_{44}$	5.13
34	60.16	Hexadecane, 2,6,11,15-tetramethyl-	$C_{20}H_{42}$	4.76
35	60.2	Heptadecane, 2,6,10,15-tetramethyl-	$C_{21}H_{44}$	6.37
36	60.25	Methanone, (1-hydroxycyclohexyl)phenyl-	$C_{13}H_{26}O_2$	87.1
37	60.3	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	$C_{15}H_{26}0$	27.2

38	60.4	Methyl tetradecanoate	$C_{15}H_{30}O_2$	53.0
39	60.46	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$	8.02
40	60.58	Pentadecane, 2,6,10,14-tetramethyl	$C_{19}H_{40}$	88.8
41	60.64	Heptacosane	$C_{27}H_{56}$	7.01
42	61.2	Octadecane	$C_{18}H_{36}$	10.3
43	61.6	Heptacosane	$C_{27}H_{56}$	7.05
44	62.1	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	$C_{16}H_{16}O_3$	14.3
46	62.2	cis-9-Hexadecenoic acid, heptyl ester	$C_{23}H_{44}O_2$	20.2
47	62.3	Heptacosane	$C_{27}H_{56}$	7.60
48	62.4	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	69.7
49	62.73	Hentriacontane	$C_{31}H_{64}$	8.02
50	62.77	1,2-Benzenedicarboxylic acid, butyl 8-methylnonyl ester	$C_{22}H_{34}O_4$	26.2
51	63.1	Eicosane	$C_{20}H_{42}$	14.1
52	63.95	Hentriacontane	$C_{31}H_{64}$	7.86
53	63.99	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	12.1
54	64.04	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	16.2
55	64.09	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	6.45
56	64.2	Hentriacontane	$C_{31}H_{64}$	7.28
57	64.3	Eicosane, 7-hexyl-	$C_{26}H_{54}$	5.52
58	64.5	Sulfurous acid, butyl octadecyl ester	$C_{22}H_{46}O_3S$	5.12
59	64.6	Hentriacontane	$C_{31}H_{64}$	8.57
60	64.8	Docosane	$C_{22}H_{46}$	7.92
61	64.9	Heptacosane	$C_{27}H_{56}$	11.5
62	65.7	Hentriacontane	$C_{31}H_{64}$	8.65
63	65.92	Heneicosane, 11-(1-ethylpropyl)-	$C_{26}H_{54}$	7.29
64	65.98	Hentriacontane	$C_{31}H_{64}$	6.99
65	66.0	2,4-Diacetyl-3-(4-chlorophenyl)-5-hydroxy-5-methylcyclohexanone	$C_{17}H_{19}CLO_4$	3.90
66	66.3	Hentriacontane	$C_{31}H_{64}$	9.67
67	67.5	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	26.2
68	67.58	Hentriacontane	$C_{31}H_{64}$	8.34
69	68.8	9-Hydroxycalabaxanthone	$C_{24}H_{24}O_6$	49.9
70	69.0	Heptacosane	$C_{27}H_{56}$	18.7
71	69.4	Octacosane	$C_{28}H_{58}$	10.3
72	69.5	Squalene	$C_{30}H_{50}$	10.6

Table 3. 2 the GC-MS analysis of Rose-yeast 1.0.

NO	RT.	Name	Formula	Prob(%)
1	50.512	Nerol	$C_{10}H_{18}O$	65.0
2	50.702	Citronellol	$C_{10}H_{20}O$	15.9
3	51.855	Geraniol	$C_{10}H_{18}O$	41.4

Table S4. Plasmids used in this work

Name	Description	Source
43802CA9	Cloning P_{TEF1}-*CAS9*-T_{CYCI} cassette into p414	Addgene
pUC57	Cloning vector with multiple cloning sites, *Amp*	GenScript
p-SINDPS1	Cloning *SINDPS1* gene into pUC57	This study
p-GmNES	Cloning *GmNES* gene into pUC57	This study
p-CbLIS	Cloning *CbLIS* gene into pUC57	This study
p-synCsVa1	Cloning *synCsVa1* gene into pUC57	This study
pM2-tHMG1	Cloning P_{PGK1}-*tHMG1*-T_{ADH1}cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM3-GES	Cloning P_{TEF1}-*tObGES*-T_{CYC1} cassette into pEASY-Blunt simple	Wu *et al.*(2)
pM3-synSmFPS	Cloning P_{TEF1}-*synSmFPS*-T_{CYC1} cassette into pEASY-Blunt simple	Zhang *et al.*(4)
pM3-ERG10	Cloning P_{TEF1}-*ERG10*-T_{CYC1} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM4-ERG8	Cloning P_{TDH3}-*ERG8*-T_{TPI1} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM4-ERG20$^{F96W-N127W}$	Cloning P_{TDH3}-*ERG20*$^{F96W-N127W}$-T_{TPI1} cassette into pEASY-Blunt simple	WU *et al.*(2)
pM4-OYE2	Cloning P_{TDH3}-*OYE2*-T_{TPI1} cassette into pEASY-Blunt simple	This study
pM4-GmNES	Cloning P_{TDH3}-*GmNES*-T_{TPI1} cassette into pEASY-Blunt simple	This study
pM5-ERG19	Cloning P_{PYK1}-*ERG19*-T_{PGI1} cassette into pEASY-Blunt simple	Lin *et al.*(1)

pM7-HMGR	Cloning P_{TEF2}-*HMGR*-T_{ENO2} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM8-ERG13	Cloning P_{FBA1}-*ERG13*-T_{TDH2} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM9-ERG12	Cloning P_{PDC1}-*ERG12*-T_{ENO2} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pM16-IDI1	Cloning P_{ENO2}-*IDI1*-T_{PDC1} cassette into pEASY-Blunt simple	Lin *et al.*(1)
pRS425-LEU2-P_{TEF1}-Pn3-32-T_{CYC1}	Containing P_{TEF1}-*Pn3-32*-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	Wang *et al.*(3)
pRS425-LEU2-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	Containing P_{TEF1}-*tObGES-ERG20**-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	This study
pRS425-LEU2-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	Containing P_{TEF1}-*ERG20**-*tObGES*-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	This study
pRS425-LEU2-P_{TEF1}-CbLIS-ERG20*-T_{CYC1}	Containing P_{TEF1}-*CbLIS-ERG20**-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	This study
pRS425-LEU2-P_{TEF1}-SINDPS1-T_{CYC1}	Containing P_{TEF1}-*SINDPS1*-T_{CYC1} cassette，CEN6/ARSH4, LEU2 marker	This study
pRS425-LEU2-P_{TEF1}-synSmFPS-synCsVa1-T_{CYC1}	Containing P_{TEF1}-*synSmFPS-synCsVa1*-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	This study
pRS313-LEU2-P_{TEF1}-synSmFPS-optiNES-T_{CYC1}	Containing P_{TEF1}-*synSmFPS-optiNES*-T_{CYC1} cassette, CEN6/ARSH4, LEU2 marker	Zhang *et al.*(4)
pNDT80-HIS3	Cloning *NDT80* and *HIS3* marker into pEASY-Blunt simple	Zhang *et al.*(4)
pGAL80-LEU2	Cloning *GAL80* and *LEU2* marker into pEASY-Blunt simple	
pGAL7-URA3	Cloning *GAL7* and *URA3* marker into pEASY-Blunt simple	Lin *et al.*(1)
pERG20gRNA	Containing pERG20 site gRNA, 2 micron, URA3 marker	This study
pERG9gRNA	Containing pERG9 site gRNA, 2 micron, URA3 marker	This study

YJL064WgRNA	Containing YJL064W site gRNA, 2 micron, URA3 marker	This study
pRS425-LEU2-TRP1-URA3-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	Containing P_{TEF1}-*tObGES*-*ERG20**-T_{CYC1} cassette, CEN6/ARSH4, LEU2, TRP1 and HIS3 marker	This study

Table S5. Strains used in this work

Name	Description	Source
CEN.PK2-1D	MATα, ura3-52; trp1-289; leu2-3,112; his3Δ1; MAL2-8C; SUC2	EUROSCARF
HP001	CEN.PK2-1D, *YJL064W::* P_{PGK1}-*tHMG1*-T_{ADH1}, P_{PDC1}-*ERG12*-T_{ADH2}, P_{ENO2}-*IDI1*-T_{PDC1}, P_{PYK1}-*ERG19*-T_{PGI1}, P_{TEF2}-*HMGR*-T_{ENO2}, P_{FBA1}-*ERG13*-T_{TDH2}, P_{TDH3}-*ERG8*-T_{TPI1} *and* P_{TEF1}-*ERG10*-T_{CYC1}, harboring plasmids p414-P_{TEF1}-43802Cas9-T_{CYC1} and pYJL064WgRNA	This study
GER-1	HP001, harboring pRS425-LEU2-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	This study
GER-2	HP001, harboring pRS425-LEU2-P_{TEF1}-ERG20*-*tObGES*-T_{CYC1}	This study
CIT1	HP001, *NDT80::HIS3*-P_{PGK1}-*tHMG1*-T_{ADH2}-P_{TDH3}-*OYE2*-T_{TPI1}-P_{TEF1}-*tObGES-ERG20**-T_{CYC1}	This study
HP001-pERG1-ERG20, HP001-pERG3-ERG20, HP001-pERG7-ERG20, HP001-pERG11-ERG20, HP001-pERG25-ERG28-ERG20	HP001, the native ERG20 promoter was replaced by pERG1, pERG3, pERG7, pERG11, pERG25, pERG28, pIDI1 and pHMG1 promoter, respectively.	This study

G20, HP001-pERG28-ERG20, HP001-pIDI1-ERG20, HP001-pHMG1-ERG20		
CIT-pERG1, CIT-pERG3, CIT-pERG7-1, CIT-pERG11, CIT-pERG25, CIT-pERG28, CIT-pIDI1, CIT-pHMG1	HP001-pERG1-ERG20, HP001-pERG3-ERG20, HP001-pERG7-ERG20, HP001-pERG11-ERG20, HP001-pERG25-ERG20, HP001-pERG28-ERG20, HP001-pIDI1-ERG20, HP001-pHMG1-ERG20, *NDT80:: HIS3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TDH3}-OYE2-T_{TPI1}-P_{TEF1}-tObGES-ERG20*-T_{CYC1},* respectively.	This study
CIT-pERG7-2	CIT-pERG7-1, pRS425-LEU2-TRP1-URA3-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	This study
NEROL	HP001, *NDT80::HIS3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TDH3}-GmNES-T_{TPI1}-P_{TEF1}-SINDPS1-T_{CYC1}*	This study
NEROL-pERG7	HP001-pERG7-ERG20, *NDT80::HIS3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TDH3}-GmNES-T_{TPI1}-P_{TEF1}-SINDPS1-T_{CYC1}*	This study
Rose–yeast	NEROL-pERG7, *GAL7::URA3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TDH3}-OYE2-T_{TPI1}-P_{TEF1}-tObGES-ERG20*-T_{CYC1}*	This study
Rose–yeast 1.0	Rose–yeast, pRS425-LEU2-TRP1-URA3-P_{TEF1}-*tObGES*-ERG20*-T_{CYC1}	This study
LIN	HP001, *NDT80::HIS3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TEF1}-CbLIS-ERG20*-T_{CYC1}*	This study
LIN-pERG7	HP001-pERG7-ERG20, *NDT80::HIS3-P_{PGK1}-tHMG1-T_{ADH2}-P_{TEF1}-CbLIS-ERG20*-T_{CYC1}*	This study
HP001-pERG7-ERG9	HP001, the native ERG9 promoter was replaced by pERG7 promoter	This Study
NED	HP001, harboring pRS313-LEU2-P_{TEF1}-synSmFPS-optiNES-T_{CYC1}	This Study

NED-pERG7	HP001-pERG7-ERG9, harboring pRS313-LEU2-P_{TEF1}-synSmFPS-optiNES-T_{CYC1}	This Study
VAL	HP001, harboring pRS425-LEU2-P_{TEF1}-synSmFPS-synCsVa1-T_{CYC1}	This Study
VAL-pERG7	HP001-ERG7-ERG9, harboring pRS425-LEU2-P_{TEF1}-synSmFPS-synCsVa1-T_{CYC1}	This study

Supplementary Table S1-S5 Reference :

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Table S6. Summary of recently achieved PVTs production in engineered *E. coli* or *S. cerevisiae.*

	Product	Organism	Carbon source	Titre	Culture conditions	Reference
Monoterpene (C10)	Linalool	*E. coli*	Glucose	505 mg/L	Shake flasks	[1]
	1,8-cineole	*E. coli*	Glucose	653 mg/L	Shake flasks	[1]
	Limonene	*E. coli*	Glycerol	2.7 g/L	**Fed-batch fermentation**	[2]
		E. coli	Glucose	600 mg/L	Shake flasks	[3]
		S. cerevisiae	Glucose	0.9 g/L	**Fed-batch fermentation**	[4]
		S. cerevisiae	Glucose	166 mg/L	Shake flasks	[5]
		S. cerevisiae	Glucose	2.6 g/L	**Fed-batch fermentation**	[6]
	β-pinene	**Cell-free**	Glucose	14.9 g/L	Cell-free systems	[7]
	Geraniol	*E. coli*	Glucose	1.12 g/L	Shake flasks	[8]
		E. coli	Glycerol	0.6 g/L	Shake flasks	[9]
		E. coli	Glucose	2.1 g/L	Shake flasks	[10]
		S. cerevisiae	Glucose	1.68 g/L	**Fed-batch fermentation**	[11]
		S. cerevisiae	Glucose	1.69 g/L	**Fed-batch fermentation**	[12]
		S. cerevisiae	Glucose	5.5 g/L	**Fed-batch fermentation**	[6]
	Sabinene	*S. cerevisiae*	Glucose	113 mg/L	Shake flasks	[5]
		Cell-free	Glucose	15.9 g/L	Cell-free systems	[7]
Sesquiterpene(C15)	β-copaene	*E. coli*	Glycerol	215 mg/L	**Fed-batch fermentation**	[13]
	Cubebol	*E. coli*	Glycerol	497 mg/L	**Fed-batch fermentation**	[13]
	Viridiflorol	*E. coli*	Glucose	25.7 g/L	**Fed-batch fermentation**	[14]
	Bisabolol	*E. coli*	Glycerol	9.1 g/L	**Fed-batch fermentation**	[15]
	Bisabolene	*S. cerevisiae*	Glucose	5.2 g/L	**Fed-batch fermentation**	[16]

Nerolidol	*S. cerevisiae*	Glucose	3.5 g/L	Shake flasks	[17]
β-Farnesene	*S. cerevisiae*	Glucose	130 g/L	**Fed-batch fermentation**	[18]
Artemisinic acid	*S. cerevisiae*	Glucose	25 g/L	**Fed-batch fermentation**	[19]

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Supplementary Sequences

Code-Optimized genes sequences (for *Saccharomyces cerevisiae*)

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