

**Debottlenecking mevalonate pathway for antimalarial drug precursor  
amorphadiene biosynthesis in *Yarrowia lipolytica***

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**Table S1. Genes used in this paper.**

| Gene           | Origin                          | Database and accession number    | Optimization | References                                          |
|----------------|---------------------------------|----------------------------------|--------------|-----------------------------------------------------|
| <i>AaADS</i>   | <i>Artemisia annua</i>          | NCBI: AEQ63683.1                 | Yes          |                                                     |
| <i>YlHMG1</i>  | <i>Yarrowia lipolitica</i> Polg | UniProtKB: Q6C704                | Yes          | (Huang et al., 2018)                                |
| <i>tYlHMG1</i> | <i>Yarrowia lipolitica</i> Polg | UniProtKB: Q6C704                | Yes          |                                                     |
| <i>SpHMG1</i>  | <i>Streptococcus pneumoniae</i> | UniProtKB - Q8DNS5               | Yes          |                                                     |
| <i>ScHMG1</i>  | <i>Saccharomyces cerviciae</i>  |                                  | Yes          | (Basson, Thorsness, & Rine, 1986)                   |
| <i>ERG13</i>   | <i>Yarrowia lipolitica</i> Polg | GRYC <sup>1</sup> : YALI0F30481g | Yes          | (Martin, Pitera, Withers, Newman, & Keasling, 2003) |
| <i>ERG8</i>    | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0E06193g               | Yes          | (Martin et al., 2003)                               |
| <i>ERG10</i>   | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0B08536g               | Yes          | (Paddon et al., 2013)                               |
| <i>ERG12</i>   | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0B16038g               | Yes          | (Martin et al., 2003)                               |
| <i>MVD1</i>    | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0F05632g               | Yes          | (Martin et al., 2003)                               |
| <i>ERG20</i>   | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0E05753g               | Yes          | (Westfall et al., 2012)                             |
| <i>POT1</i>    | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0E18568g               | Yes          |                                                     |
| <i>PAT1</i>    | <i>Yarrowia lipolitica</i> Polg | GRYC: YALI0E11099g               | Yes          |                                                     |

(1) GRYC refers to the genome resources for yeast chromosomes (<http://gryc.inra.fr/index.php?page=home>).

**Table S2. Strains constructed in this paper.**

| Strain                               | Annotation                                                                                                                       | References |
|--------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|------------|
| Po1g/ADS                             | Po1g containing pYLXP'-AaADS, used for the optimization of amorphadiene production                                               | This work  |
| Po1g/AYIH                            | Po1g containing pYLXP'-AaADS-YIHMG1, used for the optimization of amorphadiene production                                        | This work  |
| Po1g/AtYIH                           | Po1g containing pYLXP'-AaADS-tYIHMG1, used for the optimization of amorphadiene production                                       | This work  |
| Po1g/ASpH                            | Po1g containing pYLXP'-AaADS-SpHMG1, used for the optimization of amorphadiene production                                        | This work  |
| Po1g/AScH                            | Po1g containing pYLXP'-AaADS-tScHMG1, used for the optimization of amorphadiene production                                       | This work  |
| Po1g/AYIHERG8                        | Po1g containing pYLXP'- AaADS-YIHMG1-ERG8, used for the optimization of mevalonate pathway                                       | This work  |
| Po1g/AYIHERG12                       | Po1g containing pYLXP'- AaADS-YIHMG1-ERG12, used for the optimization of mevalonate pathway                                      | This work  |
| Po1g/AYIHMD1                         | Po1g containing pYLXP'- AaADS-YIHMG1-MVD1, used for the optimization of mevalonate pathway                                       | This work  |
| Po1g/AYIHERG20                       | Po1g containing pYLXP'- AaADS-YIHMG1-ERG20, used for the optimization of mevalonate pathway                                      | This work  |
| Po1g/AYIH1 <sub>x2</sub> E13E10E12   | Po1g containing pYLXP'- AaADS-YIHMG1 <sub>x2</sub> - ERG13-ERG10-ERG12 used for the optimization of amorphadiene production      | This work  |
| Po1g/AYIH1 <sub>x2</sub> E13E10E12E8 | Po1g containing pYLXP'- AaADS-YIHMG1 <sub>x2</sub> - ERG13-ERG10-ERG12-ERG8 used for the optimization of amorphadiene production | This work  |
| Po1g/A <sub>x2</sub> tYIH            | Po1g containing pYLXP'- AaADS <sub>x2</sub> -tYIHMG1 used for the optimization of amorphadiene production                        | This work  |
| Po1g/A <sub>x2</sub> tYIHE12         | Po1g containing pYLXP'- AaADS <sub>x2</sub> -tYIHMG1-ERG12 used for the optimization of amorphadiene production                  | This work  |
| Po1g/A <sub>x2</sub> tYIHP           | Po1g containing pYLXP'- AaADS <sub>x2</sub> -tYIHMG1-YIPOT1 used for the optimization of amorphadiene production                 | This work  |
| Po1g/A <sub>x2</sub> tYIHE12P        | Po1g containing pYLXP'- AaADS <sub>x2</sub> -tYIHMG1-ERG12-YIPOT1 used for the optimization of amorphadiene production           | This work  |
| Po1g/A <sub>x2</sub> tYIHPP          | Po1g containing pYLXP'- AaADS <sub>x2</sub> -tYIHMG1-YIPOT1-YIPAT1 used for the optimization of amorphadiene                     | This work  |

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|                                |                                                                                                                                |           |
|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------|
|                                | production                                                                                                                     |           |
| Polg/A <sub>x2</sub> tylHE12PP | Polg containing pYLXP' - AaADS <sub>x2</sub> -tYIHMG1-ERG12-YIPOT1-YIPAT1 used for the optimization of amorphadiene production | This work |

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**Table S3. Primers used in this paper.**

| Primer    | Sequence (5'-3')                                         |
|-----------|----------------------------------------------------------|
| YIHMGI F  | GCACTTTTTGCAGTACTAACCGCAGCTACAAGCAGCTATTGGAAAGATTGTGG    |
| YIHMGI R  | GGCCATGGAAGTAGTCGGTACCCTATGACCGTATGCAAATATTCGAACC        |
| tYIHMGI F | GCACTTTTTGCAGTACTAACCGCAGCTACGAGAAGTTGTGCGAACCCAGTCTG    |
| tYIHMGI R | GCCATGGAAGTAGTCGGTACCCTATGACCGTATGCAAATATTCGAACC         |
| SpHMG1 F  | CCAGCACTTTTTGCAGTACTAACCGCAGACAGGGAAAACCGGCCATATAG       |
| SpHMG1 R  | ACAGGCCATGGAAGTAGTCGGTACCTCATGTATTTTCCAGAACCTGCTTC       |
| tScHMG1 F | AGCACTTTTTGCAGTACTAACCGCAGGACCAGCTTGTTAAAACAGAAGTA       |
| tScHMG1 R | GACAGGCCATGGAAGTAGTCGGTACCTTATGATTTTATGCAGGTTACTGA       |
| ERG13 F   | CCGACCAGCACTTTTTGCAGTACTAACCGCAGTCGCAACCCCAGAACGTTGG     |
| ERG13 R   | GGACAGGCCATGGAAGTAGTCGGTACCCTATGACGAACTAGAGCATGAAAG      |
| ERG10 F   | CCGACCAGCACTTTTTGCAGTACTAACCGCAGGAGCCCGTCTACATTGTTTCTAC  |
| ERG10 R   | GGACAGGCCATGGAAGTAGTCGGTACCCTAACACTTCTCAACAATGATAGA      |
| ERG8 F    | CCGACCAGCACTTTTTGCAGTACTAACCGCAGACCACCTATTCGGCTCCGGGAAAG |
| ERG8 R    | GGACAGGCCATGGAAGTAGTCGGTACCCTACTTGAACCCCTTCTCGAGCCG      |
| ERG12 F   | CCGACCAGCACTTTTTGCAGTACTAACCGCAGGACTACATCATTTTCGGCGCCGAG |
| ERG12 R   | GGGACAGGCCATGGAAGTAGTCGGTACCCTAATGGGTCCAGGGACCGATG       |
| MVD1 F    | CCGACCAGCACTTTTTGCAGTACTAACCGCAGATCCACCAGGCCTCCACCACCGC  |
| MVD1 R    | GGACAGGCCATGGAAGTAGTCGGTACCCTACTTGCTGTTCTTCAGAGAACC      |
| ERG20 F   | CCAGCACTTTTTGCAGTACTAACCGCAGGCTTCAGAAAAAGAAATTAGGAGAG    |
| ERG20 R   | GGACAGGCCATGGAAGTAGTCGGTACCCTATTTGCTTCTCTTGTAAC          |
| YIPOT1 F  | CCGACCAGCACTTTTTGCAGTACTAACCGCAGGACCGACTTAACAACCTCGCC    |
| YIPOT1 R  | GGACAGGCCATGGAAGTAGTCGGTACTTACTCGGCAACAACCAGAGAAGC       |
| YIPAT1 F  | GACCAGCACTTTTTGCAGTACTAACCGCAGCGACTCACTCTGCCCCGACTTAAC   |
| YIPAT1 R  | GGACAGGCCATGGAAGTAGTCGGTACCTACTCGACAGAAGAGACCTTCTTG      |

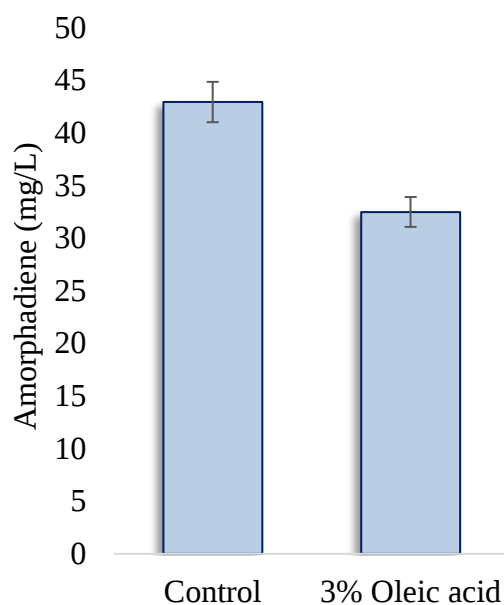
**Table S4. Plasmids used in this paper.**

| Plasmid                                                    | Annotation                                                                              |
|------------------------------------------------------------|-----------------------------------------------------------------------------------------|
| pYLXP'                                                     | YaliBrick plasmid, used for pathway assemble                                            |
| pYLXP'-AaADS                                               | For the construction and optimization of amorphadiene production                        |
| pYLXP'-AaADS-YIHMG1                                        | For the construction and optimization of amorphadiene production                        |
| pYLXP'-AaADS-tYIHMG1                                       | For the construction and optimization of amorphadiene production                        |
| pYLXP'-AaADS-SpHMG1                                        | For the construction and optimization of amorphadiene production                        |
| pYLXP'-AaADS-tSchMG1                                       | For the construction and optimization of amorphadiene production                        |
| pYLXP'- AaADS-YIHMG1-ERG8                                  | For the construction and optimization of mevalonate pathway                             |
| pYLXP'- AaADS-YIHMG1-ERG12                                 | For the construction and optimization of mevalonate pathway                             |
| pYLXP'- AaADS-YIHMG1-MVD1                                  | For the construction and optimization of mevalonate pathway                             |
| pYLXP'- AaADS-YIHMG1-ERG20                                 | For the construction and optimization of mevalonate pathway                             |
| pYLXP'- AaADS-YIHMG1 <sub>x2</sub> -ERG13-ERG10-ERG12      | For the construction and optimization of mevalonate pathway and amorphadiene production |
| pYLXP'- AaADS-YIHMG1 <sub>x2</sub> -ERG13-ERG10-ERG12-ERG8 | For the construction and optimization of mevalonate pathway and amorphadiene production |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1 <sup>1</sup>          | For the construction and optimization of amorphadiene production                        |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1-ERG12                 | For the construction and optimization of amorphadiene production                        |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1-ylPOT1                | For enhancing acetyl-CoA synthesis and improvement of amorphadiene production           |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1-ERG12-ylPOT1          | For enhancing acetyl-CoA synthesis and improvement of amorphadiene production           |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1-ylPOT1-ylPAT1         | For enhancing acetyl-CoA synthesis and improvement of amorphadiene production           |
| pYLXP'- AaADS <sub>x2</sub> -tylHMG1-                      | For enhancing acetyl-CoA synthesis and improvement                                      |

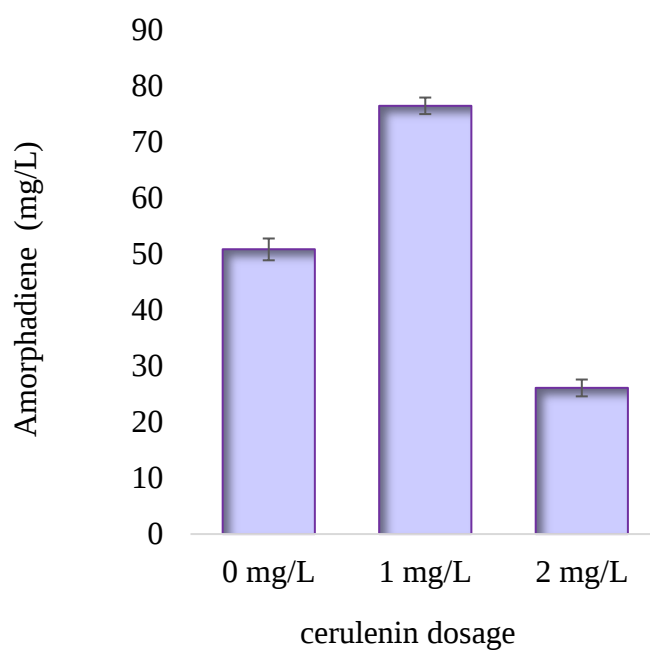
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(1) The subscripts “<sub>x2</sub>” refer to gene copy number in the plasmids.

## Supplementary figures



**Supplementary Figure S1.** Effect of 3% oleic acid on amorphadiene production carried out for Po1g/AYIH. CSM-Leu media without Oleic acid used as a control condition



**Supplementary Figure S2.** Cerulenin optimization dosage carried out for Po1g/AYIH strain in leucine drop-out complete synthetic media (CSM-Leu).

## References

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