**Additional File 1**

**Metabolic engineering of *Escherichia* *coli* for production of mixed isoprenoid alcohols and their derivatives**

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**Table S1.** Primers, plasmids, and bacterial strains used in this study.

**Table S2.** Time course analysis of oxidation of farnesol to farnesal in strain DH5α-YjgB.

**Figure S1.** GC-FID and GC-MS profile of standard isoprenoid-based alcohols and their derivatives.

**Figure S2.** Comparison of the cell growth of strains NA-MBF2.0, NAK-MBF2.0, NA-MBF1.0, and NAK-MBF1.0.

**Figure S3.** Comparison of the cell growth of strains NA-MBF1.1, NA-MBF1.2, NA-MBF1.1a, and NA-MBF1.2a

**Figure S4.** Percent composition of isoprenoid biofuels obtained from strains NA-MBF1.1, NA-MBF1.2, NA-MBF1.1a, and NA-MBF1.2a.

**Figure S5.** GC-FID standard curves of isoprenoid-based alcohols and their derivatives.

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| --- | --- | --- |
| **Names** | **Descriptions** | **References** |
| **Primersa** |  |  |
| IspA-F | CTAGGAGCTCAGCCCTAAGGAACCAATATGGACTTTCCGCAGCAAC | This study |
| IspA-R | TGAGGTACCTTATTTATTACGCTGGATGATGTAG | This study |
| NudB-F1 | CTGGATCCTTTAAGAAGGAGATATACATATGAAGGATAAAGTGTATAAGCGTCC | This study |
| NudB-R1 | CCCAAGCTTAGGCAGCGTTAATTACAAACTG | This study |
| IspA\*-F | GAGGTACCAACAATAATCTCGTATAGTATGGACTTTCCGCAGCAAC | This study |
| IspA\*-R | CTGGATCCTTATTTATTACGCTGGATGATGTAG | This study |
| NudB-F2 | CATCCATGGTTTAAGAAGGAGATATACATATGAAGGATAAAGTGTATAAGCGTCC | This study |
| NudB-R2 | CATGGAGCTCTTAGGCAGCGTTAATTACAAACTG | This study |
| AphA-F | TATCGTCGACAAGTCATTCGGGGCCAATTTATGCGCAAGATCACACAGGC | This study |
| AphA-R | CCCAAGCTTAGTATTCTGAATTGACGATCACCTC | This study |
| Idi-F | ACGGATCCTGAGGAGGTAACGTATGCAAACGGAACACGTCATTTTA | This study |
| Idi-R | TATCGTCGACTCTAAGATCTTATTTAAGCTGGGTAAATGCAG | This study |
| AdhE-F | TACGAATTCAGGAGGTAATAATAATGGCTGTTACTAATGTCGC | This study |
| AdhE-R | ACGGATCCTAAAGCGGATTTTTTCGC | This study |
| **Plasmids** |  |  |
| pSTV28 | P*lac* expression vector, pACYC184 origin, lacZ, Cmr | Takara Co., Ltd |
| pSNA | pSTV28 containing *mvaE* and *mvaS* of *E. faecalis,* *mvaK1, mvaK2,* and *mvaD* of *S. pneumoniae,* and *idi* of *E. coli* | [[1](#_ENREF_1)] |
| pSTV28K | P*lac* expression vector, pACYC184 origin, lacZ, Kmr | [[2](#_ENREF_2)] |
| pSNAK | pSTV28K containing *mvaE* and *mvaS* from *E. faecalis*, *mvaK1, mvaK2*, and *mvaD* from *S. pneumoniae*, and idi from *E. coli* | [[2](#_ENREF_2)] |
| pTrc99A | P*trc* expression vector, pBR322 origin, lacIq, Ampr | [[3](#_ENREF_3)] |
| pT-SBL | pTrc99A vector containing *ispA* from *E. coli* | This study |
| pT-MBF1.0 | pTrc99A vector containing *ispA* and *nudB* from *E. coli* | This study |
| pT-MBF2.0 | pTrc99A vector containing *ispA*\* and *nudB* from *E. coli* | This study |
| pT-MBF1.1 | pTrc99A vector containing *nudB* and *ispA* from *E. coli* | This study |
| pT-MBF1.2 | pTrc99A vector containing *nudB, ispA* and *aphA* from *E. coli* | This study |
| pT-MBF1.1a | pTrc99A vector containing *nudB, ispA* and *idi* from *E. coli* | This study |
| pT-MBF1.2a | pTrc99A vector containing *nudB, ispA*, *idi* and *aphA* from *E. coli* | This study |
| pT-YahK | pTrc99A vector containing *yahK* from *E. coli* | [[2](#_ENREF_2)] |
| pT-YddN | pTrc99A vector containing *yddN* from *E. coli* | [[2](#_ENREF_2)] |
| pT-AdhE | pTrc99A vector containing *adhE* from *E. coli* | This study |
| pT-YjgB | pTrc99A vector containing *yjgB* from *E. coli* | [[2](#_ENREF_2)] |
| **Strains** |  | [[2](#_ENREF_2)] |
| MG1655 | *E. coli* K-12; F- lambda-, ilvG-, rfb-50, rph-1 | ATCC 700926b |
| DH5α | *E. coli* K-12; F–, Φ80lacZΔM15, Δ(lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rK–, mK+) phoA, supE44, λ–,thi-1 | ATCC 98040 |
| NA-MBF1.0 | *E. coli* DH5α harboring pT-MBF1.0 and pSNA | This study |
| NAK-MBF1.0 | *E. coli* DH5α harboring pT-MBF2.0 and pSNA | This study |
| NA-MBF2.0 | *E. coli* DH5α harboring pT-MBF1.0 and pSNAK | This study |
| NAK-MBF2.0 | *E. coli* DH5α harboring pT-MBF2.0 and pSNAK | This study |
| NA-MBF1.1 | *E. coli* DH5α harboring pT-MBF1.1 and pSNA | This study |
| NA-MBF1.2 | *E. coli* DH5α harboring pT-MBF1.2 and pSNA | This study |
| NA-MBF1.1a | *E. coli* DH5α harboring pT-MBF1.1a and pSNA | This study |
| NA-MBF1.2a | *E. coli* DH5α harboring pT-MBF1.2a and pSNA | This study |
| DH5α-Trc | *E. coli* DH5α harboring pTrc99A | This study |
| DH5α -YjgB | *E. coli* DH5α harboring pT-YjgB | This study |
| DH5α -Yahk | *E. coli* DH5α harboring pT-Yahk | This study |
| DH5α -Yddn | *E. coli* DH5α harboring pT-YddN | This study |
| DH5α -Adhe | *E. coli* DH5α harboring pT-AdhE | This study |
| MG*ΔYjgB* | MG1655 *ΔYjgB* | [[2](#_ENREF_2)] |

**Table S1.**

**Primers, plasmids, and bacterial strains used in this study.**

aRestriction enzyme sites are underlined.

bATCC, American type culture collection.

**Table S2.**

**Time course analysis of oxidation of farnesol to farnesal in strain DH5α-YjgB.**

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| --- | --- | --- | --- |
| Time (h) | Farnesol (mg/L) | Farnesal (mg/L) | Total (mg/L) |
| 12 | 979 ± 5.6 | 10 ± 1.5 | 989 ± 6.6 |
| 24 | 956 ± 6.3 | 28 ± 2.6 | 984 ± 8.9 |
| 36 | 896 ± 9.1 | 89 ± 7.2 | 985 ± 16.3 |
| 48 | 873 ± 7.1 | 116 ± 4.2 | 989 ± 11.3 |

\*Farnesol was fed in a two-phase culture of strain DH5α-YjgB at a concentration of 1 g/L. The culture was initially induced with 0.2 mM IPTG and incubated at 30°C with 250 rpm shaking in a rotary shaker for 48 h. Decane phase was collected at 12 h intervals and subsequently subjected to GC for analysis of compositional variations between farnesol and farnesal. Results are presented as the means of the triplicate experiments (M ± SD).

**Figure S1.**

**GC-FID and GC-MS profile of standard isoprenoid alcohols and their derivatives.** GC chromatograms **(A)** and mass spectra **(B)** of standard isoprenoid compounds. Standard citral exists as isomers, *E*-citral (f1) and *Z*-citral (f2). Standard farnesal also exists as isomers of *Z*,*E*-farnesal (i1) and *E*,*E*-farnesal (i2).

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**Figure S2.**

**Comparison of cell growth of strains NA-MBF2.0 and NAK-MBF2.0 (upper panel) and the strains NA-MBF1.0 and NAK-MBF1.0 (lower panel).** Strains NA-MBF2.0 and NAK-MBF2.0 are *E. coli* DH5α harboring pSNA and pSNAK, respectively, in addition to pT-MBF2.0; strains NA-MBF1.0 and NAK-MBF1.0 harbor pT-MBF1.0 instead of pT-MBF1.0. Strains were cultured at 30°C for 48 h in 2YT medium containing 2.0% (v/v) glycerol and were initially induced with 0.5 mM IPTG. Error bars represent the range from three independent experiments.

**Figure S3.**

**Comparison of cell growth of strains NA-MBF1.1, NA-MBF1.2, NA-MBF1.1a, and NA-MBF1.2a harboring NA-MBF1.1, NA-MBF1.2, NA-MBF1.1a, and NA-MBF1.2a, respectively, in addition to pSNA.** The dotted line represents the cell growth of strain NA-MBF1.0. The strains were cultured at 30°C for 48 h in 2YT medium containing 2.0% (v/v) glycerol and were initially induced with 0.5 mM IPTG. The error bars represent the range from three independent experiments.

**Figure S4.**

****Percent composition of isoprenoids mixtures obtained from strains NA-MBF1.1, NA-MBF1.2, NA-MBF1.1a, and NA-MBF1.2a. The strains NA-MBF1.1 and NA-MBF1.2 have no additional overexpression of IDI, while the strains NA-MBF1.1a and NA-MBF1.2a have additional overexpression of IDI. Strains were cultured at 30°C for 48 h in 2YT medium containing 2.0% (v/v) glycerol and were initially induced with 0.5 mM IPTG.

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**Figure S5.**

**GC-FID standard curves of isoprenoid alcohols and their derivatives.** All standard curves were generated using the means of three replicated experiments. The x-axis represents the GC peak area of the isoprenoids, while the y-axis represents the concentration of the corresponding standard compound in ethyl acetate.

**References**

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