

Metabolic engineering of *Escherichia coli* for production of non-natural acetins from glycerol

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Table S1. List of plasmids and bacterial strains used in this study

Names	Descriptions	Sources
Plasmids		
pSTV28	P _{lac} expression vector, pACYC184 origin, lacZ α , Cm ^r	Takara Co. , Ltd
pTrc99A	P _{trc} expression vector, pBR322 origin, lacI ^q , Amp ^r	Amersham Bioscience
pET28a (+)	P _{T7} expression vector, pBR322 origin, lacI, and Kan ^r	Novagen
pET30a	P _{T7} expression vector, pBR322 origin, lacI, and Kan ^r	Novagen
pET30a-Ec-maa	pET30a vector containing <i>maa</i> from <i>E. coli</i>	This study
pMD1	pTrc99A vector containing <i>lacA</i> from <i>E. coli</i>	This study
pMD2	pTrc99A vector containing <i>cysE</i> from <i>E. coli</i>	This study
pMD3	pTrc99A vector containing <i>yjgM</i> from <i>E. coli</i>	This study
pMD4	pTrc99A vector containing <i>yjaB</i> from <i>E. coli</i>	This study
pMD5	pTrc99A vector containing <i>yjiD</i> from <i>E. coli</i>	This study
pMD6	pTrc99A vector containing <i>wecH</i> from <i>E. coli</i>	This study
pMD7	pTrc99A vector containing <i>nhoA</i> from <i>E. coli</i>	This study
pMD8	pTrc99A vector containing <i>maa</i> from <i>E. coli</i>	This study
pMD9	pTrc99A vector containing <i>maa</i> from <i>S. carnosus</i>	This study
pMD10	pTrc99A vector containing <i>maa</i> from <i>H. jeotgali</i>	This study
pMD11	pTrc99A vector containing <i>maa</i> from <i>L. brevis</i>	This study
pMD12	pTrc99A vector containing <i>maa</i> from <i>P. putida</i>	This study
pMD13	pTrc99A vector containing <i>maa</i> from <i>B. subtilis</i>	This study
pMD14	pET28a vector containing <i>maa</i> from <i>B. subtilis</i>	This study
pT-CAT	pTrc99A vector containing <i>cat</i> from pSTV28	This study
pMDT1	pSTV28 vector containing <i>maa</i> from <i>B. subtilis</i>	This study
pMDT2	pTrc99A vector containing <i>cat</i> from pSTV28 and <i>maa</i> from <i>B. subtilis</i>	This study
pMDT3	pTrc99A vector containing <i>oat</i> from <i>B. cereus</i> and <i>maa</i> from <i>B. subtilis</i>	This study
pMDT4	pTrc99A vector containing <i>oat</i> from <i>P. aeruginosa</i> and <i>maa</i> from <i>B. subtilis</i>	This study
pMDT5	pTrc99A vector containing <i>oat</i> from <i>C. acetobutylicum</i> and <i>maa</i> from <i>B. subtilis</i>	This study
pMDT6	pTrc99A vector containing <i>oat</i> from <i>M. abscessus</i> and <i>maa</i> from <i>B. subtilis</i>	This study
pMDT7	pTrc99A vector containing <i>oat</i> from <i>L. brevis</i> and <i>maa</i> from <i>B. subtilis</i>	This study
Strains		
MG1655	<i>E. coli</i> K-12; F ⁻ lambda ⁻ , ilvG ⁻ , rfb-50, rph-1	ATCC 700926
DH5 α	<i>E. coli</i> K-12; F ⁻ , Φ 80lacZ Δ M15, Δ (lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rK ⁻ , mK ⁺) phoA, supE44, λ ⁻ , thi-1	ATCC 98040
BW25113	Δ (araD-araB)567, Δ lacZ4787(::rrnB-3), lambda ⁻ , rph-1, Δ (rhaD-rhaB)568, hsdR514	CGSC 7636

AceCo W3110	MG1655 <i>ΔackA-pta, poxB, ldhA, dld, adhE, pps, atoDA</i> <i>E. coli</i> K-12; F ⁻ , λ ⁻ IN (rrnD-rrnE)1	¹ ATCC 27325
DH5α (DE3)	<i>E. coli</i> K-12; F ⁻ , Φ80lacZΔM15, Δ(lacZYA-argF)U169, deoR, recA1, endA1, hsdR17(rK ⁻ , mK ⁺) phoA, supE44, λ ⁻ , thi-1, λ(DE3)	This study
DH-MD0	<i>E. coli</i> DH5α harboring pTrec99A	This study
JM109	<i>E. coli</i> K-12; e14–(McrA–) recA1 endA1 gyrA96 thi-1 hsdR17 (rK – mK ⁺) supE44 relA1 Δ(lac-proAB) [F traD36 proAB lacIqZΔM15]	This study
BL21(DE3) T1 ^R	<i>E. coli</i> K-12; F-ompT hsdSB(rB- mB-) gal dcm l(DE3) tonA	This study
DH-MD1	<i>E. coli</i> DH5α harboring pMD1	This study
DH-MD2	<i>E. coli</i> DH5α harboring pMD2	This study
DH-MD3	<i>E. coli</i> DH5α harboring pMD3	This study
DH-MD4	<i>E. coli</i> DH5α harboring pMD4	This study
DH-MD5	<i>E. coli</i> DH5α harboring pMD5	This study
DH-MD6	<i>E. coli</i> DH5α harboring pMD6	This study
DH-MD7	<i>E. coli</i> DH5α harboring pMD7	This study
DH-MD8	<i>E. coli</i> DH5α harboring pMD8	This study
DH-MD9	<i>E. coli</i> DH5α harboring pMD9	This study
DH-MD10	<i>E. coli</i> DH5α harboring pMD10	This study
DH-MD11	<i>E. coli</i> DH5α harboring pMD11	This study
DH-MD12	<i>E. coli</i> DH5α harboring pMD12	This study
DH-MD13	<i>E. coli</i> DH5α harboring pMD13	This study
DH-MD14	<i>E. coli</i> DH5α(DE3) harboring pMD14	This study
DH-MDT1	<i>E. coli</i> DH5α harboring pMDT1	This study
DH-CAT	<i>E. coli</i> DH5α harboring pT-CAT	This study
DH-MDT2	<i>E. coli</i> DH5α harboring pMDT2	This study
DH-MDT3	<i>E. coli</i> DH5α harboring pMDT3	This study
DH-MDT4	<i>E. coli</i> DH5α harboring pMDT4	This study
DH-MDT5	<i>E. coli</i> DH5α harboring pMDT5	This study
DH-MDT6	<i>E. coli</i> DH5α harboring pMDT6	This study
DH-MDT7	<i>E. coli</i> DH5α harboring pMDT7	This study
AC-MDT2	<i>E. coli</i> AceCo harboring pMDT2	This study
BW-MDT2	<i>E. coli</i> BW25113 harboring pMDT2	This study
W3-MDT2	<i>E. coli</i> W3110 harboring pMDT2	This study
MG-MDT2	<i>E. coli</i> MG1655 harboring pMDT2	This study

Table S2. Data collection and refinement statistics of Ec-MAA in complex with Tris.

Ec-MAA with Tris	
Data collection	
Space group	<i>P3</i>
Cell dimensions	
a, b, c (Å)	62.10,62.10,81.13
α , β , γ (°)	90.00,90.0,120.0
Resolution (Å)	50.00-1.34(1.36-1.34)
R _{sym} or R _{merge}	6.8(32.5)
I / σ (I)	34.7 (3.2)
Completeness (%)	99.5 (96.2)
Redundancy	5.6 (3.5)
Refinement	
Resolution (Å)	50.00-1.34
No. reflections	78711
R _{work} / R _{free}	17.1/19.0
No. atoms	3121
Protein	2808
Ligand/ion	16
Water	297
B-factors	15.88
Protein	15.04
Ligand/ion	15.63

Water	23.83
R.m.s. deviations	
Bond lengths (Å)	0.147
Bond angles (°)	1.820

Table S3. Codon optimized heterologous genes for expression in *E. coli*

Genes	Codon optimized sequences (5'-3')
<i>Sc-maa</i>	ATGACCACCGAGAAGGAAAAAATGCTGAGCGGTCAGCTGTACAACAGCCGTGACCCGCA ACTGGTTAAGGAGCGTCACAAAGCGCGTCACGCGACCAAGGCGATCAACAACGCGTTCA GCATTAAAGAACGTCACCTTTCTGCTGCGTCAGAGCATCGGTCACTGCGGCGACAACGTG TTCATCGAGCCGGACATTCACTTCGATTACGGTTACAACATTAGCCTGGGCAACCACTT CTATGCGAACTTTAACCCGGTTATGCTGGATGTGGCGCCGATCACCATTGGTAACCACG TTCTGCTGGGCCCGAACGTGCAACTGATCACCAGCGACCCACCCGCTGAACCCGGCGGAG CGTGCGAGCGGTCTGGAAGTGGCGTTCCCGATCATGATTGGCGACCACGTTTGGATTGG TGCGGGTGCGATTGTTCTGCCGGGTGTGACCATTGGCGATAACGTGGTTGTGGGTGCGG GCAGCGTTGTGACCAAGGACATCCCGGATAACCAGGTTGTGGCGGGCAACCCGGCGCGT TTTATTTCGTGAAGTGCCGCTGGATTAA
<i>Hj-maa</i>	ATGACCAGCGAGAAGGAACGTATGCTGCGTGGTGAGCCGTACGACGCGAGCGATAGCGA GCTGGTTGCGGAACGTGAGCAGCGCGTGAAGTGAACCGTAAATATAACCGTACCACCG CGACCGAGAGCGACCGTCGTGAACGTCTGCTGGAGGAAGTGTTCGGTAGCGTGGAGGAC GCGACCGTTGAACCGCCGATCCGTTGCGATTACGGTTATAACGTTACGTTGGCGAGAA CTTCTACGCGAACTTTGACTGCGTTATTCTGGATGTGCGTCGTGTTGAATTTGGCACCC GTTGCCTGCTGGGTCCGGGTGTTACGTTTATAACCGCGACCCACCCGCTGGATGCGGAG GAACGTGCGGCGGGTCTGGAGAGCGGTGCGCCGGTGACCGTTGGTGACGATGTGTGGAT CGGTGGCCGTGCGGTTCTGAACAGCGGCGTGAGCGTTGGTGACGGCAGCGTGATTGCGA CGGTGCGGTGGTTACCGAAGATGTTCCGGCGGGCGCGCTGGTTGGTGGAACCCGGCG CGTGTGCTGAAGGACGTTGATTAA
<i>Lb-maa</i>	ATGGACAAGAGCGAGAAGGAAAAAATGATCACCGGCGAGCTGTTCAACGTGTACGATCC GGAGCTGGTTGCGGAACGTGGCGCGGCGCGTCAGCAAGTGAGGCGCTGAACGAGCTGG GTGAACAGGACCCGGAAGAGCCAGCAACTGGTTAAACGTCTGTTTCGGCGCGACCGGT GACCAAGTTGAGGTTACGCGACCTTTCTGTTGCGATTACGGCTATAACATCTACGTGGG TGAAAACCTCTTTGCGAACTATGACTGCACCATTTCTGGATGTTGCGCCGATCCGTATTG GCAAGCACTGCCTGCTGGGTCCGAAAGTGCAGATTTACAGCGTTAACCATCCGGCGGAG CCGGAAGTGCCTGTAACGGCGCGATGGGCATTGGCAAGCCGGTGACCTGGGTGACGA TGTGTGGGTTGGTGGCGGTGCGATCATCTGTCCGGGTGTGACCCTGGGTGATAACGTGA TCGTTGCGGCGGGCGCGGTGGTTACCAAGAGCTTCGGTAGCAACGTGGTTCTGGGCGGT AACCCGGCGCGTGTGATTAAAGCCGCTGAAATAA
<i>Pp-maa</i>	ATGAGCCTGAGCGAGAAGCACAAAATGCTGACCGGTCAACTGTATCATGCGGGTTGCCC GGAGCTGCAAGCGGAACAAATTGCGAACAAGCACTGGATGCACCGTTATAACAACAGCG

	TGGAAGTCTGCTGAACGACGCGCGTACGGTCTGCTGGTTGAGCACTTCGGCCAAGTGGGT GAAGGCGCGGTTATTTCGTCCGCCGTTTTACTGCGACTACGGTTATAACATCAGCGTGGG CCGTAACACCTTCATGAACTTTAACTGCGTGATCCTGGATGTGGTTCCGGTTCGTATCG GTGACGATTGCCAGATTGGCCCGAACGTGCAAATCTATACCGCGGACCACCCGCTGGAC CCGGAAGTGCCTCGTAGCGGTCTGGAAAGCGGCCGTACCGTGACCATTGGTGACAACGT TTGGATCGGTGGCGCGGCGATCATTCTGCCGGGTGTGACCATCGGCGACAACGCGATTG TTGGTGCGGGCAGCGTGGTTACCCGTGATGTTCCGGCGGGTGCGACCGTGGTTGGTAAC CCGGCGCGTGTTTCGTACGCCGGATCAGGGTCAATAA
<i>Bs-maa</i>	ATGCTGCGTACCGAGAAGGAAAAAATGGCGGCGGGTGAGCTGTACAACAGCGAAGACCA GCAACTGCTGCTGGAGCGTAAGCACGCGCGTCAGCTGATCCGTCAATATAACGAAACCC CGGAAGACGATGCGGTGCGTACCAAGCTGCTGAAAGAACTGCTGGGTAGCGTGGGCGAT CAGGTTACCATCCTGCCGACCTTCCGTTGCGACTACGGTTATCACATCCACATTGGCGA TCACACCTTCGTGAACTTTGACTGCGTTATTCTGGATGTGTGCGAGGTTTCGTATCGGCT GCCACTGCCGTGATTGCGCCGGGTGTTACATCTACACCGGGCCACCCGCTGGACCCCG ATTGAGCGTAAGAGCGGTAAAGAATTTGGCAAACCGGTGACCATTGGTGATCAGGTTTG GATCGGTGGCCGTGCGGTGATTAATCCGGGTGTGACCATCGGCGACAACGCGGTGATTG CGAGCGGCAGCGTGGTTACCAAGGATGTTCCGGCGAACACCGTGGTTGGTGGAACCCG GCGCGTATTCTGAAACAACCTGTAA
<i>Bc-Oat</i>	ATGGACTTCCACCAGATCGACATTGATCGTTGGAACCGTAAGCCGTACTTTGAGCACTA TATCAAGGAAGGTAAGTGCAGCTACAGCGTGACCGCGAACCTGAACGTTACCGCGCTGC TGAACGGCCTGCGTAAGAAAAACATGAAGCTGTACCCGGCGTTTCATCTATATGATTAGC CGTGTGGTTAACAGCCACATCGAGTTCCGTACCGCGTTTAAAGATAAGGGTCAGCTGGG CTACTGGGAACAAATGGTGCCGACCTATACCATTTTCCACAAAGAGGACAAAACCTTTA GCGCGATGTGGACCGAATACAGCAGCGATTTTCAGCCTGTTTTACAAGAACTACCTGCAA GACATCGATCGTTATGGTGACAAGAAAGGCCTGTGGGTGAAAGAGAACGTTCCGGCGCA CACCTTCAGCATTAGCGCGCTGCCGTGGGTAGCTTCACCGGTTTTCAACTGAACCTGT ACAACGGCGAGTATCTGCTGCCGATCATTACCCTGGGCAAGTACTTTAGCGATGGCGAA ACCGTGTGCCTGCCGATCAGCCTGCAAGTTCACCACGCGGTTTGCGACGGTTATCACGT TAGCATGTTTCATTAACGAACCTGCAAAAACCTGGCGGATAGCTTTGAGGAATGGCTGTAA
<i>Pa-Oat</i>	ATGAGCTACACCCGTGTTGACATCAGCAGCTGGAACCGTCGTGAGCACTTTGAAATTTT CCGTGGTGATGGCCAATGCACCTTTAGCCAGACCGTTCAACTGGACATCACCCGTCTGC TGGATTTACCCGTAGCCGTGGTTACCGTTTTATCCGGTGTTTCATCCACAGCATTGCG AAGGTGGTTAACCGTTTTCCCGAGTACCGTATGGCGATGAAAGGCGACGAACGATTGTT GTGGGATTGCGTTACCCGAACCTATACCACCTTTACCCGGACACCGAAACCTTTAGCA GCTTCTGGAGCCACTACCACGACGATCTGGCGCGTTTTCTGGCGGAGTATAGCATGGAC CGTGAAAAGTACCGTAACGATCACAGCTATTTTCCGAAAGGTTTCATCGAAAACGTGTT TTATGTTAGCGCGAACCCGTGGGTAGCTTCACCAGCTTCGATTTTAACTTCGCGAGCG CGAACAACTTCTTTGCGCCGCTGTTTACCGTGGGCAAGTACTATAGCCAAGCGGGTAAA ACCCTGGTGCCGCTGGCGGTGCAAGTTCACCATGCGGTTTGCGATGGTTTTTCATGCGGC GCGTCTGGTGACCGAGCTGCAAAAGCTGTGCGATGAAGCGGGTGGCTAA
<i>Ca-Oat</i>	ATGAACAGCAACTTCCACGCGATCGACATGAACACCTACCCGCGTGCGCACACCTACAA CTATTTTACCAAGACCGTGAGCACCTGATCTATAGCATCAACATTACCCTGGATGTTA CCATTCTGCGTGCGACCCCTGAAGAACAAGGTCTGAAATTCTTTCCGGCGTACGTGTAT CTGGTTACCCGTGCGATCGGTGCTCACCAGGAGTTCTGATGGCGATTCAAAACGACAT GCTGGGCTACTGGGATTGCCGTACCCCGTTCTATCCGATCTTTACAGGAGACGATAAGA CCATTACCTTTCTGTGGACCGAGTACAACGAAGACTTCGAGGTGTTTTACAAGAACTAC ATCAGCGATATTCTGTGAATACGGTAACAACCACGGCATCATGCTGAGCAAGGACGCTCC GCCGAGCAACAACCTACATCATTAGCTGCATTCCGTGGTTTCAGCTTTAACAGCCTGAGCA TGCAGCTGCAAAACGCGAAAACTACTATGCGCCGATCTTCGAAGCGGGTCGTTTTACC GAAACCAACGGCATCATTACCCTGCCGCTGAGCATCACCGTGAACCACGCGACCGTTGA CGGTACCACATTAAGCTGCTGCTGGATGAGCTGCAATGGAGCATGAGCCACCCGAAAG AGTGGATTTCGTTAA
<i>Ma-Oat</i>	ATGCCGGCGGAGCACGCGCTGCTGGTTGTGATGGCGACCTTCGAACCGCTGGACCTGCA ACAATGGCCGCGTCGTGAGTGGTTTTGAACACTACCTGGATCGTTGCCCGACCTACTATA GCATGACCGTGGACCTGGACATCACCAACCTGCGTGCGGCGGTTGACGCGAGCGGTGCT AAGACCTATCCGACCCAGATTTGGGCGCTGGCGACCGTGGTTAACCGTCACCCGGAGTT CCGTATGGCGCTGGACGATCAAGGTAATCCGGGTGTGTGGGACGTGGTTGATCCGGCGT TCACCGTTTTTAAACCCGGACCGTGAAACCTTTGCGGGTATCAGCGCGCGTTACACCCG GACTTCGATGCGTTTTACAGCGAGGCGGCGGAACCTGCTGAGCGAATATCGTAACGCGAG

	CACCCTGTTCCCGCAGGGCGCGCCGCGTCCGCGTAACGTGTTTGACATCAGCAGCATTC CGTGGACCCGTTTACCAGGTTTTACCCTGACCATTGCGCCGGGTTGGGCGCACCTGGCG CCGATCTTCACCATTGGTAAATTTTACGAGCAGGAAGGCCGTACCATGATGCCGCTGGC GCTGCAAATTCACCATGCGGCGGCGGACGGCTATCACAGCGCGCTCTGGTGGAGGAAC TGCGTCAAATCATTGCGGACCCGGATTGGGTTCGTGATTAA
<i>Lb-Oat</i>	ATGACCGAGCTGAACACCCATGCGACCCCGATTGACCAGACCACCTGGGCGCGTCGTGA ATACTTCTACTACTTCACCAAGATGATGCCGACCGGTTTACCCTGAACGTGGACATGG ACATCACCGCGACCAAAGCGTGGCTGACCAAGCACGATCGTAAATTCAACCCGGCGTAC CTGTATCTGGTTACCAAGCTGATTACCAAACACCCGGAGTTTCGTGTGGGTTCAGGTTGA CGGCCAACTGGTGAATACGATGTTCTGCACCCGAGCTATAGCACCTTTACGGTGACG ATCTGACCATTAGCAACCTGTGGACCGCGTATGACCCGAGCTTCGAGCAGTTTCACCAA AACTACCTGGCGGACCAAGCGACCTATGGCGATCAACATAACCCGATGCCGAAGGCGCC GCAAGGTGCGAACACCTACATGATCGGCAGCATTCCGTGGACCCACTTCAACAGCTATA CCCCGCTGCCGTTTACCCGCTGAACACCTTCTTTCCGGTGATCCAGGCGGGTCGTTTC ACCTTTAAAGATGGCCAATGGCTGATGCCGCTGAGCTTCACATTACACACGCGGTGGC GGACGGCTACCACGTTAGCCAGTTCTTTAACGAGCTGCAACGTGTTTTTGATCACCCGG AACTGTGGCTGACCGCGTAA

Table S4. List of PCR primers sequences used in this study.

Primers ^{a, b, c}	Descriptions (5'-3')	Sources
Ec-lacA-F	CTGGATCCAGGAGGTAATAAAATGGAACATGCCAATGACCG	This study
Ec-lacA-R	GTTTCTAGATTAAACTGACGATTCAACTTTATAATC	This study
Ec-cysE-F	CTGGATCCAGGAGGTAATAAAATGTCGTGTGAAGAACTGGAAAT TG	This study
Ec-cysE-R	GTTTCTAGATTAGATCCCATCCCCATACTC	This study
Ec-yjgm-F	CTGGATCCAGGAGGTAATAAAATGAATAACATTGCGCCGC	This study
Ec-yjgm-R	GTTTCTAGATTAGAGTTCGCGCAACATCC	This study
Ec-yjaB-F	CTGGATCCAGGAGGTAATAAAATGGTTATTAGTATTCGCCGCTC	This study
Ec-yjaB-R	GTTTCTAGATTACGCCCCCACATACGC	This study
Ec-yiiD-F	CTGGATCCAGGAGGTAATAAAATGAGCCAGCTTCCAGGG	This study
Ec-nhoA-F	CTGGATCCAGGAGGTAATAAAATGACGCCATTCTGAATCAC	This study
Ec-nhoA-R	GTTTCTAGATTATTTTCCCGCCTCCGGG	This study
Ec-wecH-F	CTGGATCCAGGAGGTAATAAAATGCAGCCCAAATTTACTGG	This study
Ec-Wech-R	GTTTCTAGATTAACTCACTAATCTGTTTCTGTCTG	This study
Ec-yiiD-R	GTTTCTAGATTACTCTTCTTCGTTCCCGC	This study
Ec-maa-F	CTGGATCCAGGAGGTAATAAAATGAGCACAGAAAAAGAAAAGAT G	This study
Ec-maa-R	GTTTCTAGATTACAATTTTTTAATTATTCTGGCTG	This study
Sc-maa-F	CTGGATCCAGGAGGTAATAAAATGACCACCGAGAAGGAAAAAAT G	This study
Sc-maa-R	GTTTCTAGATTAAATCCAGCGGCACTTCAC	This study
Hj-maa-F	CTGGATCCAGGAGGTAATAAAATGACCAGCGAGAAGGAACG	This study
Hj-maa-R	GTTTCTAGATTAAATCAACGTCCTTCAGCACA	This study
Lb-maa-F	CTGGATCCAGGAGGTAATAAAATGGACAAGAGCGAGAAGG	This study
Lb-maa-R	GTTTCTAGATTATTTTACGCGGCTTAATCAC	This study
Pp-maa-F	CTGGATCCAGGAGGTAATAAAATGAGCCTGAGCGAGAAGCAC	This study
Pp-maa-R	GTTTCTAGATTATTGACCCTGATCCGGCTG	This study
Bs-maa-F	CTGGATCCAGGAGGTAATAAAATGCTGCGTACCGAGAAGG	This study

Bs-maa-R	GTTTCTAGATTACAGTTGTTTCAGAATACGCGC	This study
Bs-maa-R1	ACGTCGACTTACAGTTGTTTCAGAATACGCGC	This study
Cat-F	CTAGGAGCTC <i>AGGAGAAATATA</i> ATGGAGAAAAAATCACTGGAT ATAC	This study
Cat-R	CTGGATCCTTACGCCCCGCCCTGCC	This study
Trc-Bs.maa-F	AGATCT AGAGTCGACAGTATCGGCGGG	This study
Trc-Bs.maa-R	TATATTTCTCCT GAGGATCCCCGGGTACCG	This study
Bc-Oat-F	CTCGGTACCCGGGGATCCT <i>CAGGAGAAATATA</i> ATGGACTTCCA CCAGATC	This study
Bc-Oat-R	CCCGCCGATACT GTTCGACAGATCTTACAGCCATTCCTCAAAG	This study
Ca-Oat-F	CTCGGTACCCGGGGATCCT <i>CAGGAGAAATATA</i> ATGAACAGCAA CTTCCAC	This study
Ca-Oat-R	CCCGCCGATACT GTTCGACAGATCTTAACGAATCCACTCTTTC	This study
Lb-Oat-F	CTCGGTACCCGGGGATCCT <i>CAGGAGAAATATA</i> ATGACCGAGCT GAACACCC	This study
Lb-Oat-R	CCCGCCGATACT GTTCGACAGATCTTACGCGGTCAGCCACAG	This study
Ma-Oat-F	CTCGGTACCCGGGGATCCT <i>CAGGAGAAATATA</i> ATGCCGGCGGA GCACGCG	This study
Ma-Oat-R	CCCGCCGATACT GTTCGACAGATCTTAATCACGAACCCAATCCG GGTCCG	This study
Pa-Oat-F	CTCGGTACCCGGGGATCCT <i>CAGGAGAAATATA</i> ATGAGCTACAC CCGTGTTG	This study
Pa-Oat-R	CCCGCCGATACT GTTCGACAGATCTTAGCCACCCGCTTCATC	This study
30a-Ec-maa-F	GCGCGCATATGAGCACAGAAAAAGAAAAGATGATT	This study
30a-Ec-maa-R	GCGCGCTCGAGCAATTTTTTAATTATTCTGGCTGGATTAC	This study

^aRestriction enzyme sites are underlined.

^bRBS sequences are italic.

^cThe overlapping sequences are bolded.

Table S5. Chemical formula, molar mass, and density of glycerol and acetins

Compounds	Chemical Formula	Molar mass (g/mol)	Density (g/cm³)
Glycerol	C ₃ H ₈ O ₃	92.09	1.26
Monoacetin	C ₅ H ₁₀ O ₄	134.13	1.20
Diacetin	C ₇ H ₁₂ O ₅	176.17	1.17
Triacetin	C ₉ H ₁₄ O ₆	218.21	1.15

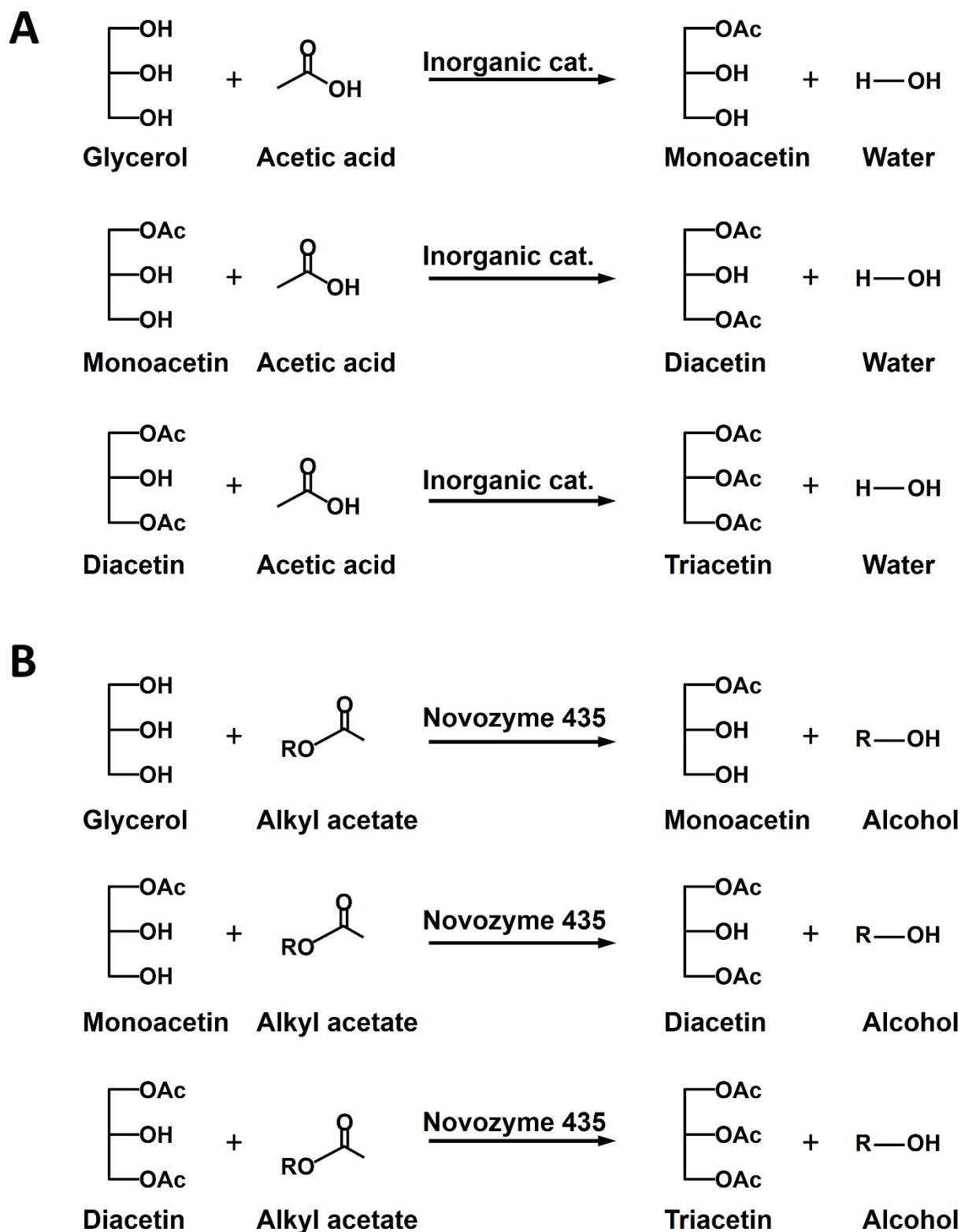


Figure S1. Schemes of acetins synthesis by chemical (A) and enzymatic (B) methods.

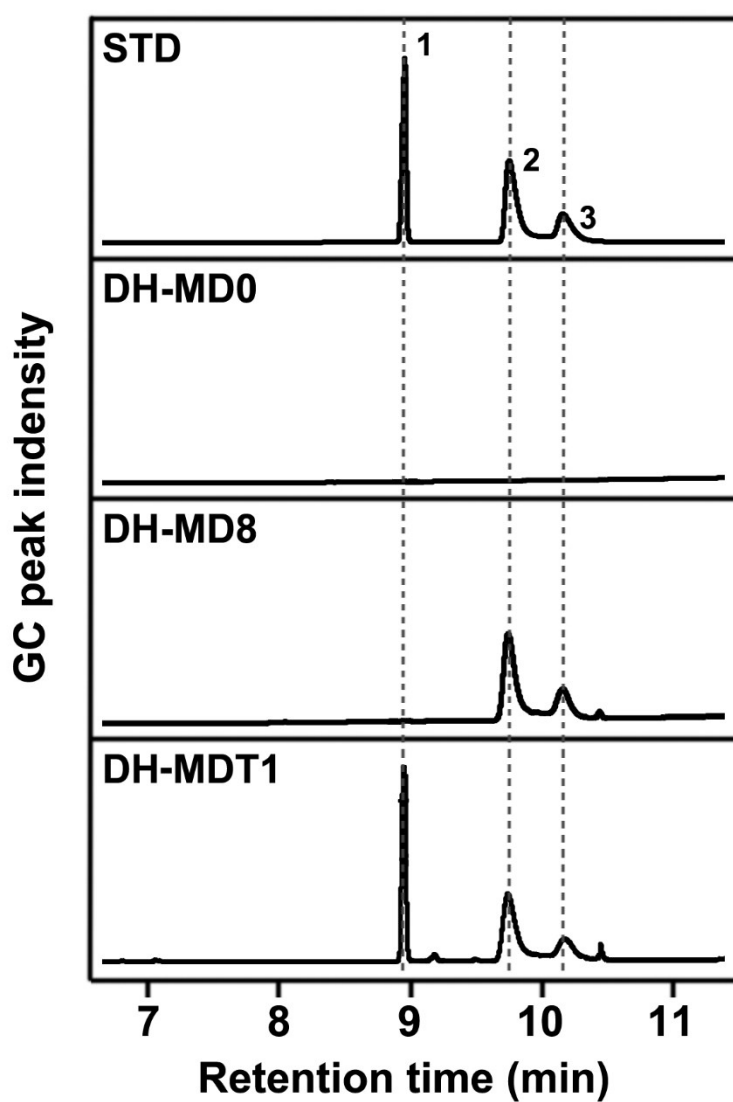


Figure S2. Chromatograms of GC analysis. GC chromatograms of the standard mixture (STD), consisting of triacetin (1), diacetin (3), and monoacetin (2), and the ethyl acetate extracts of the cultures of DH-MD0, DH-MD8 and DH-MDT1 strains.

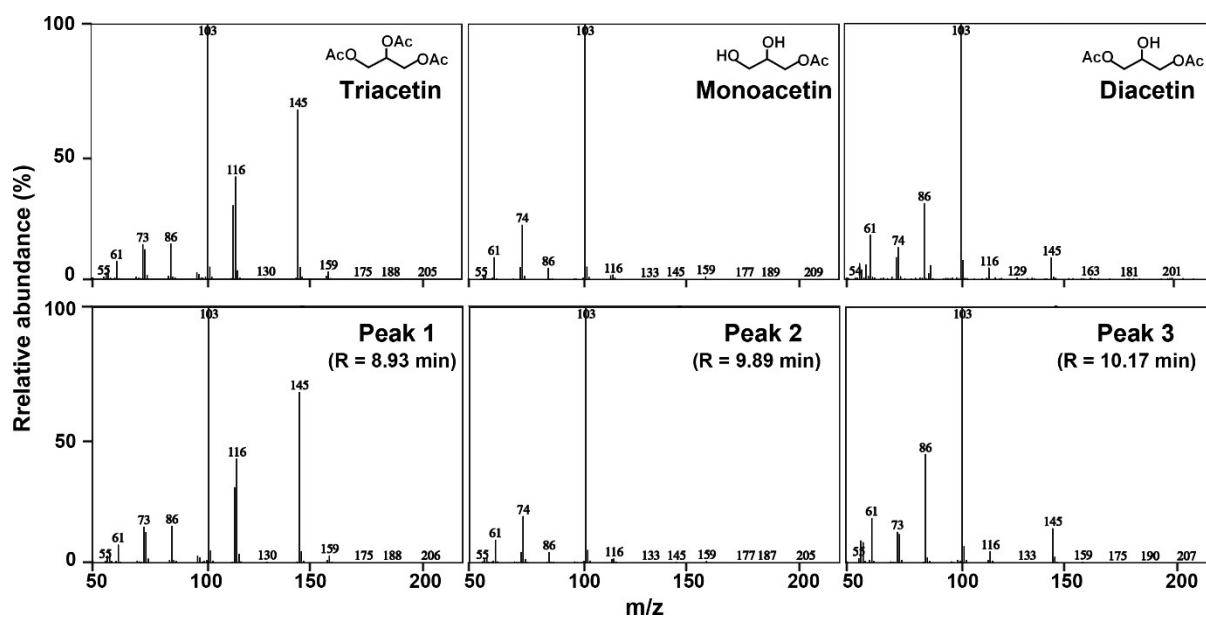


Figure S3. Chromatograms of GC-MS analysis. GC-MS chromatograms of the compounds indicated with numbered peaks in Fig. S2 and the corresponding standard compounds.

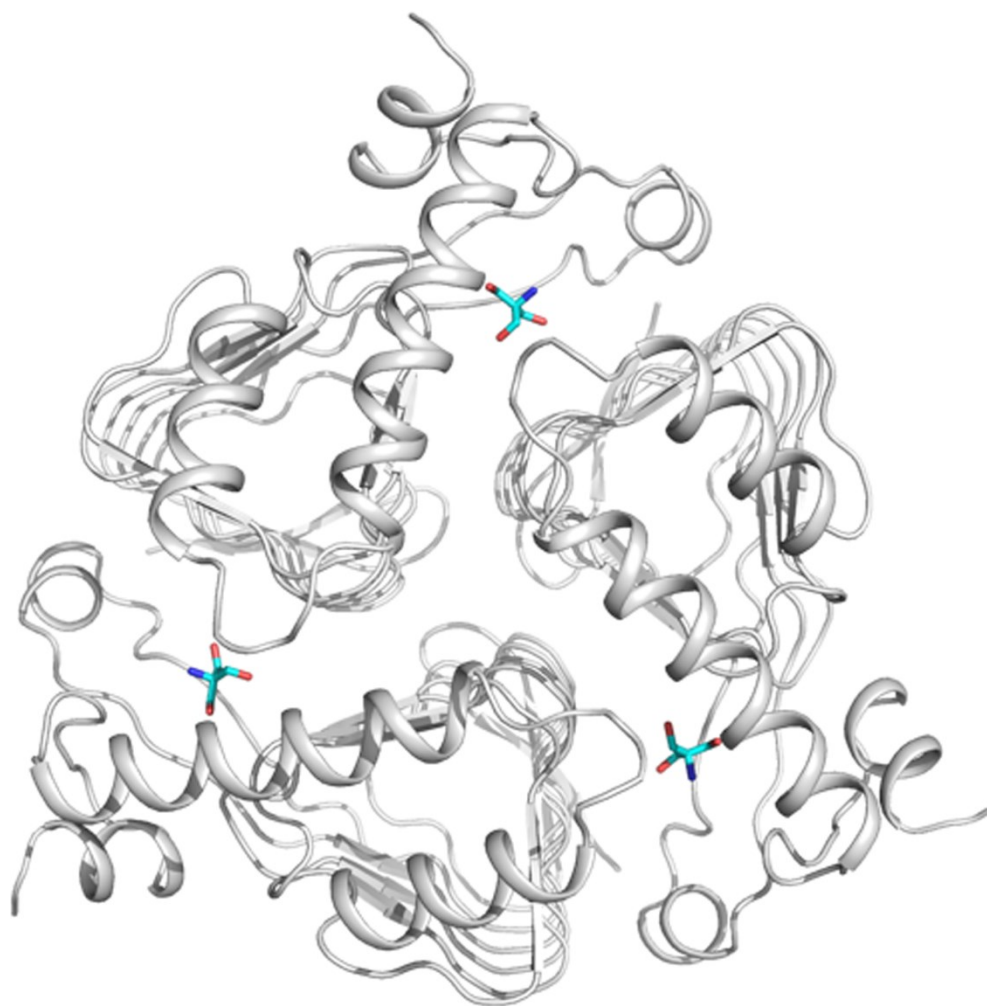


Figure S4. Trimeric structure of Ec-MAA in complex with Tris. The trimeric structure of Ec-MAA is shown as a cartoon diagram and the bound Tris molecules are as a stick model with a cyan color.

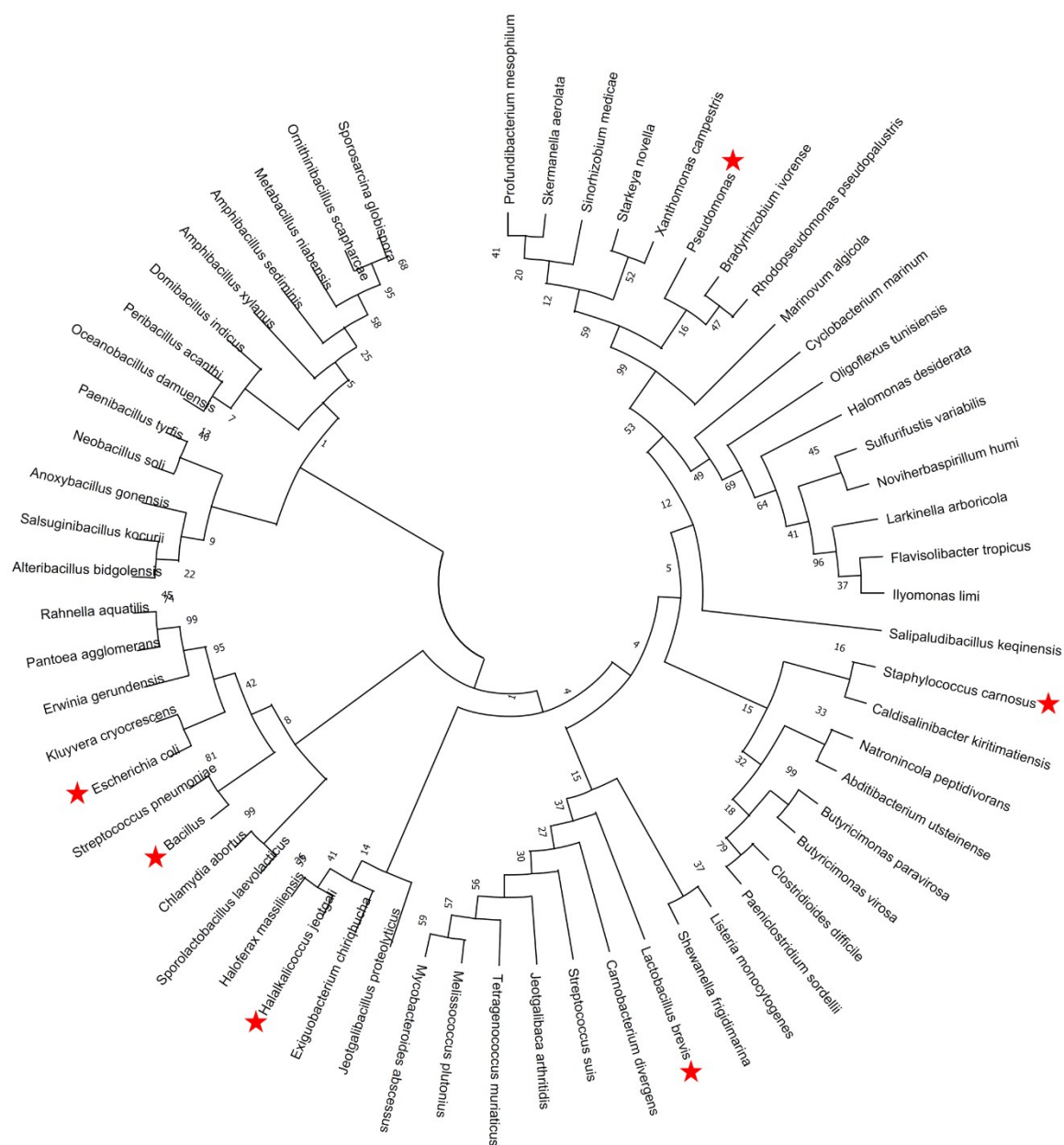


Figure S6. Phylogenetic tree analysis of O-acetyltransferases. Unrooted Maximum Likelihood tree of the O-acetyltransferase enzymes. The phylogenetic tree was drawn as a circle model. Bootstrap values are shown at each node as percentage of 100 replicates. The enzymes used in this study are indicated by red-colored stars.

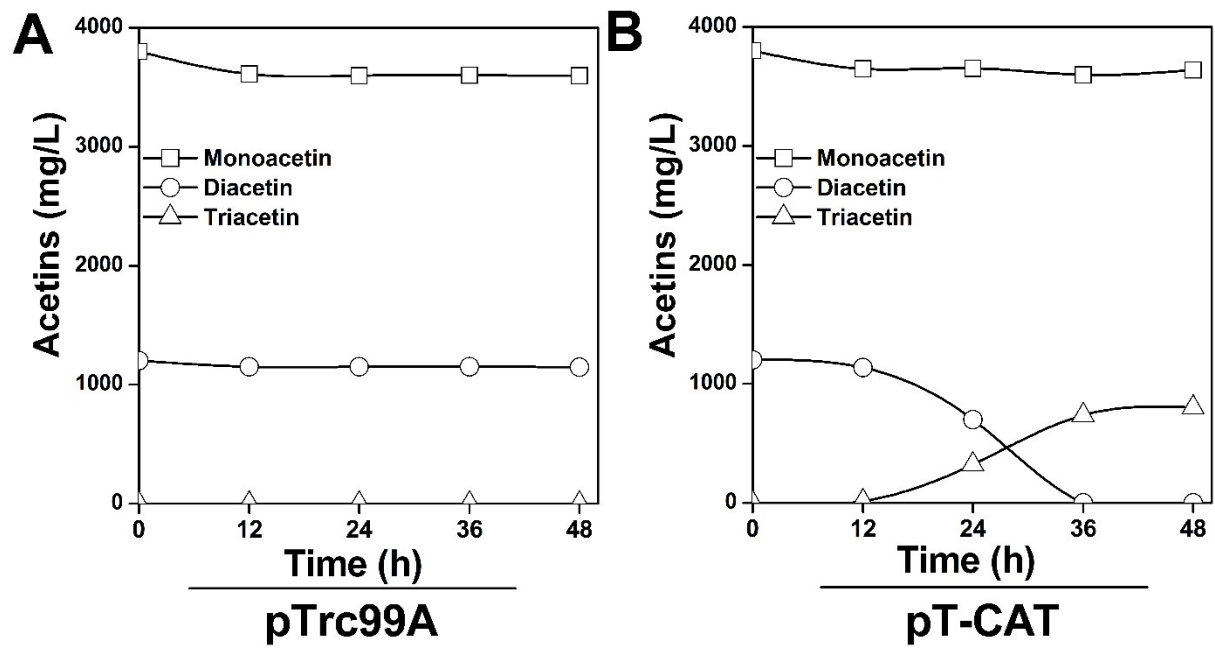


Figure S7. Feeding a mixture of mono- and diacetin to culture of the strains DH-MD0 (A) and DH-CAT (B) harboring pTrc99A and pT-CAT plasmids, respectively.

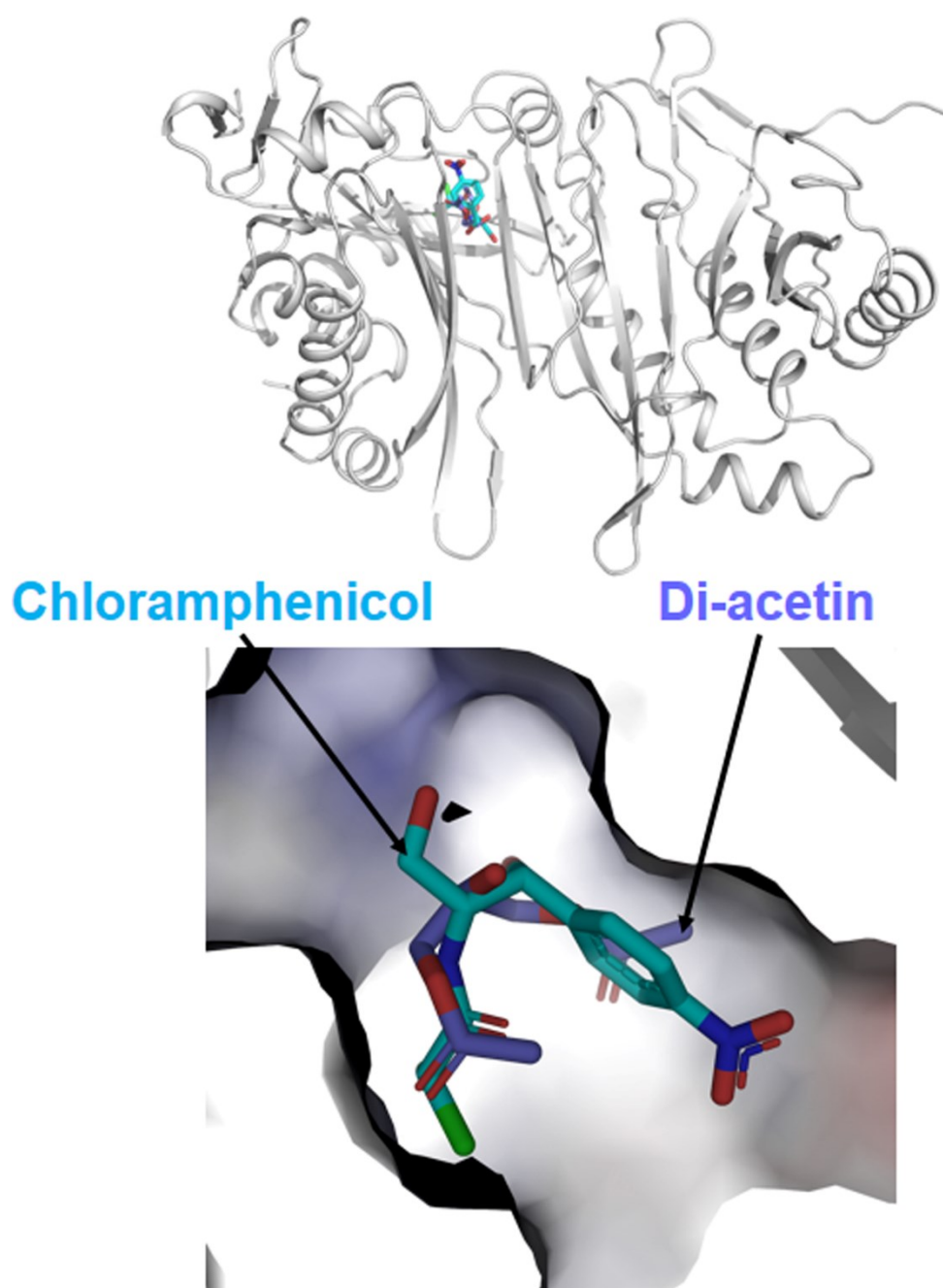


Figure S8. Overall structure of CAT. The CAT structure is shown as a cartoon (top figure) and an electrostatic potential surface (bottom figure) models, and the bound chloramphenicol and the simulated di-acetin molecules are with a stick model with colors of cyan and light-blue, respectively.

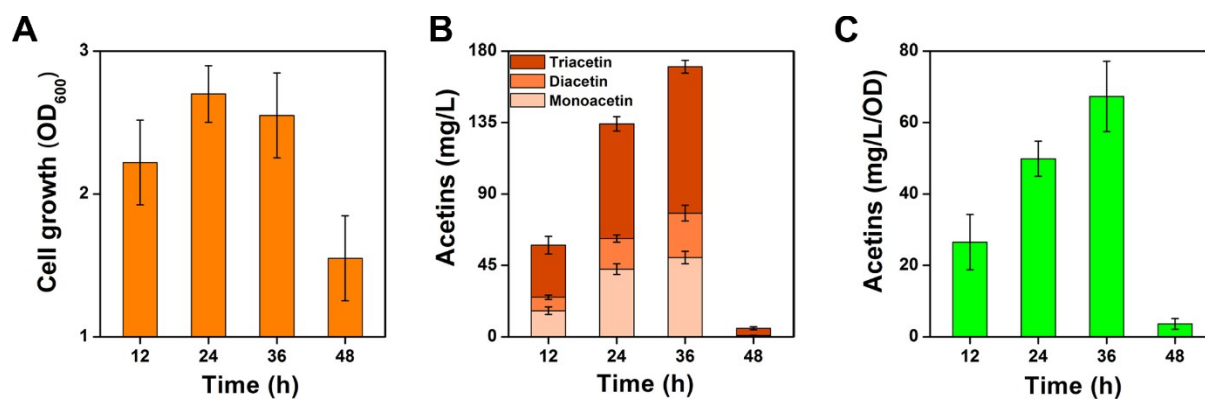


Figure S9. Evaluation of cellular activities. The cell pellets from the culture of DH-MDT2 strain are collected at 12 h interval, resuspended to an OD₆₀₀ of 1 in the fresh M9 medium with 2% (v/v) glycerol, and incubated for 12 h at 37°C with 250 rpm shaking. The means and standard divisions are from two biological replicates.

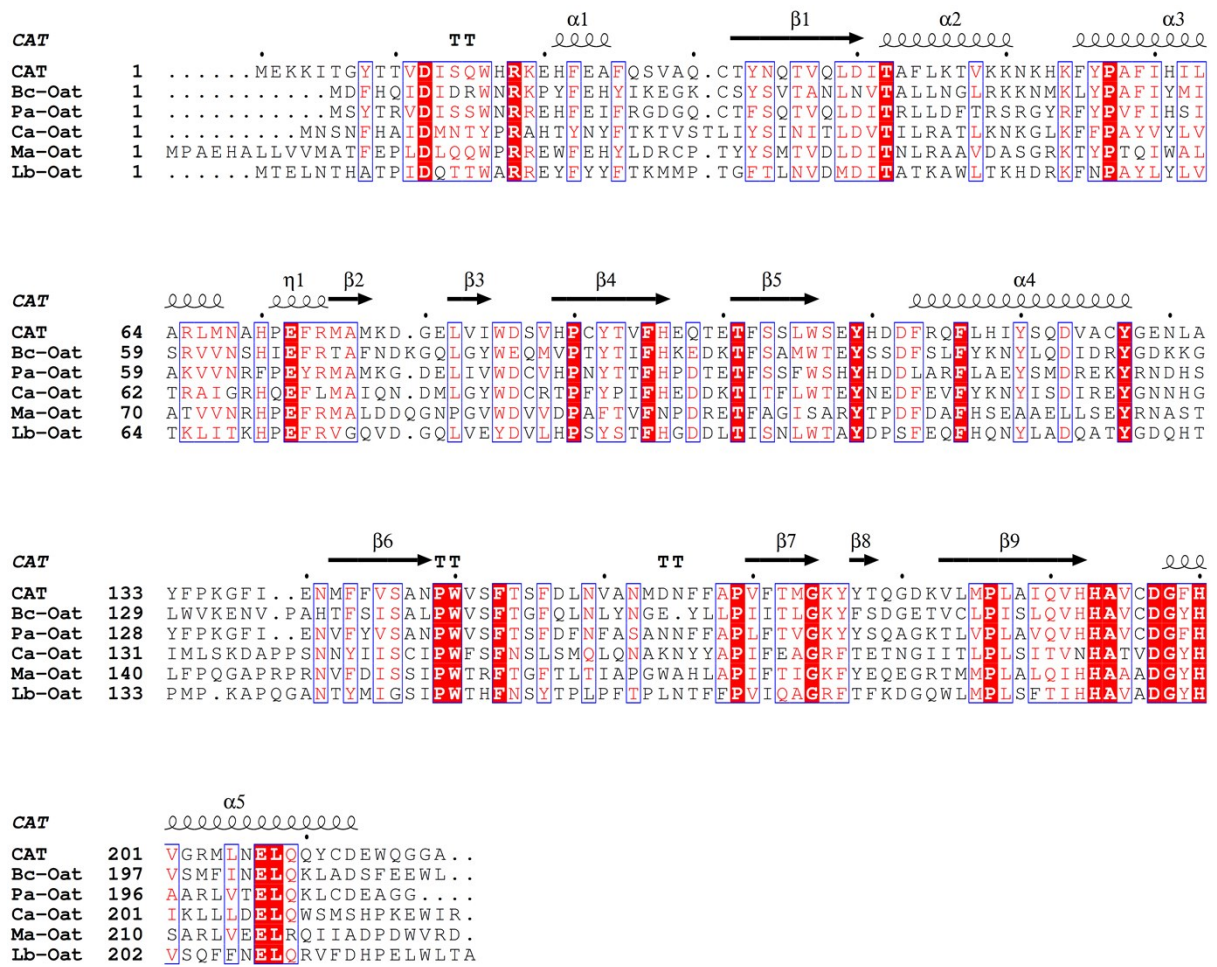


Figure S10. Multiple sequence alignment of *E. coli* CAT-like enzymes.

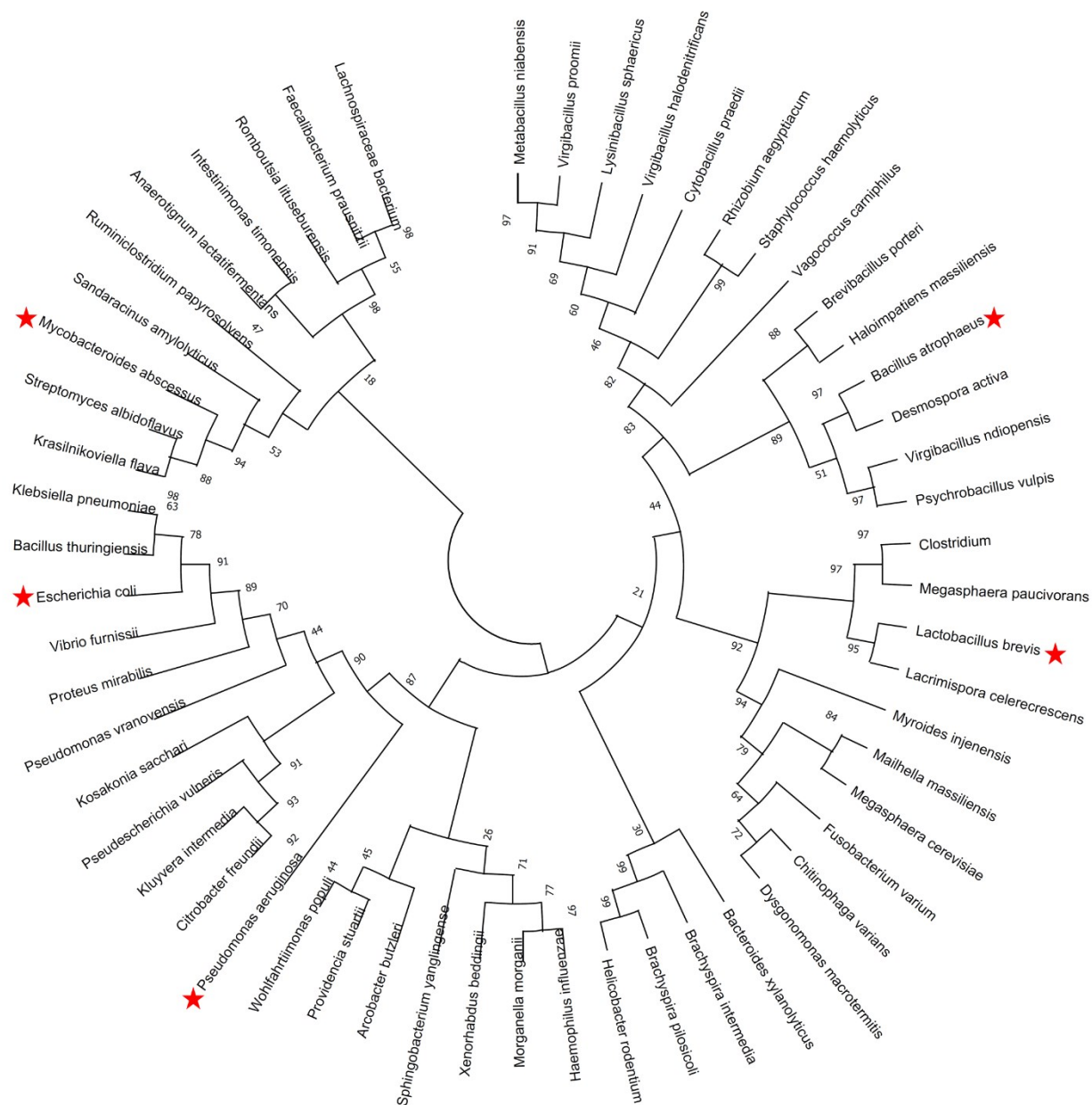


Figure S11. Phylogenetic tree analysis of Chloramphenicol O-acetyltransferases. Unrooted Maximum Likelihood tree of the Chloramphenicol O-acetyltransferases. The phylogenetic tree was drawn as a circle model. Bootstrap values are shown at each node as percentage of 100 replicates. The enzymes used in this study are indicated by red-colored stars.

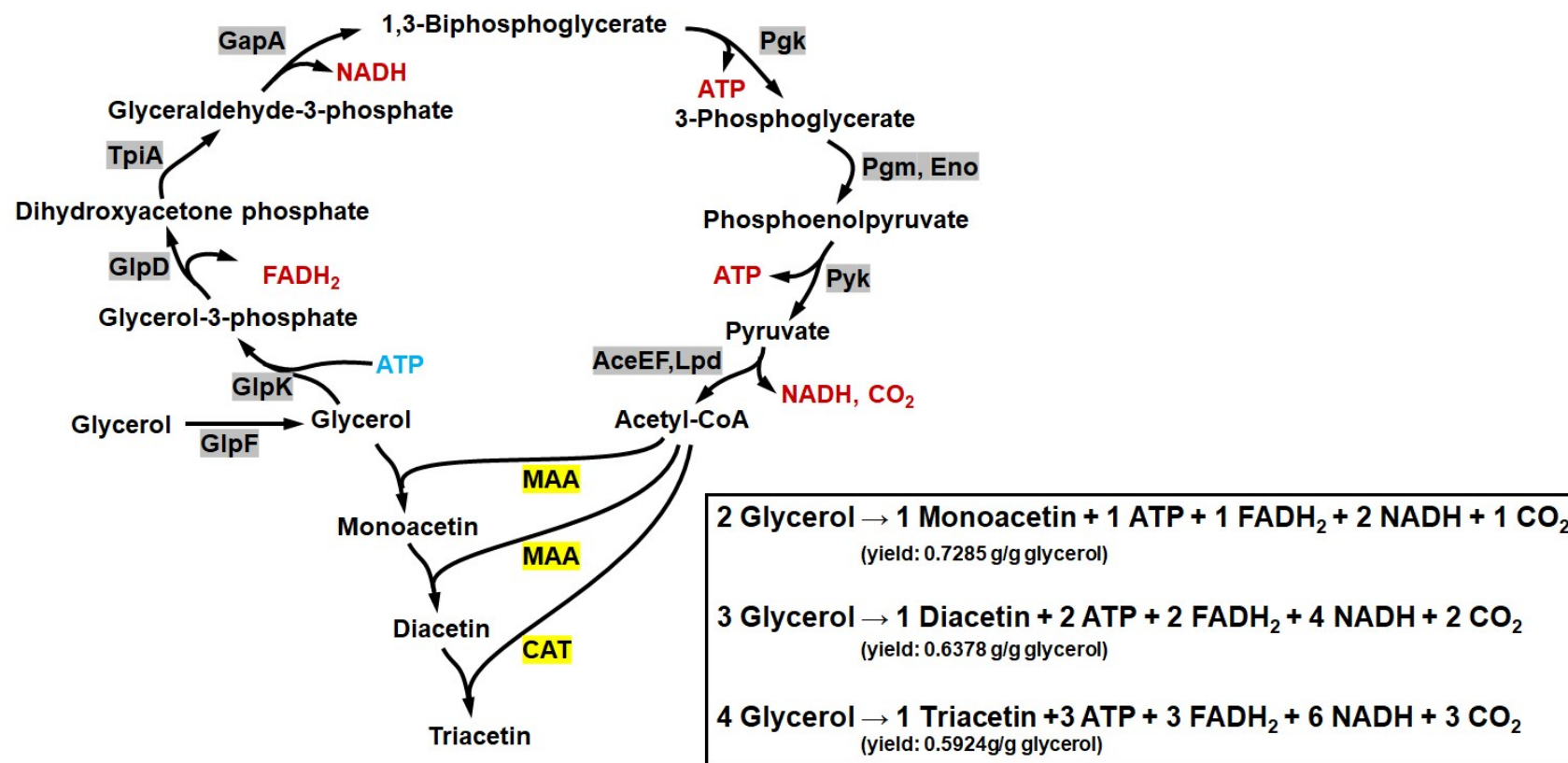


Figure S12. Proposed glycerol metabolic pathway for acetins biosynthesis and balanced chemical equations for calculation of theoretical yields of acetins in *E. coli*.

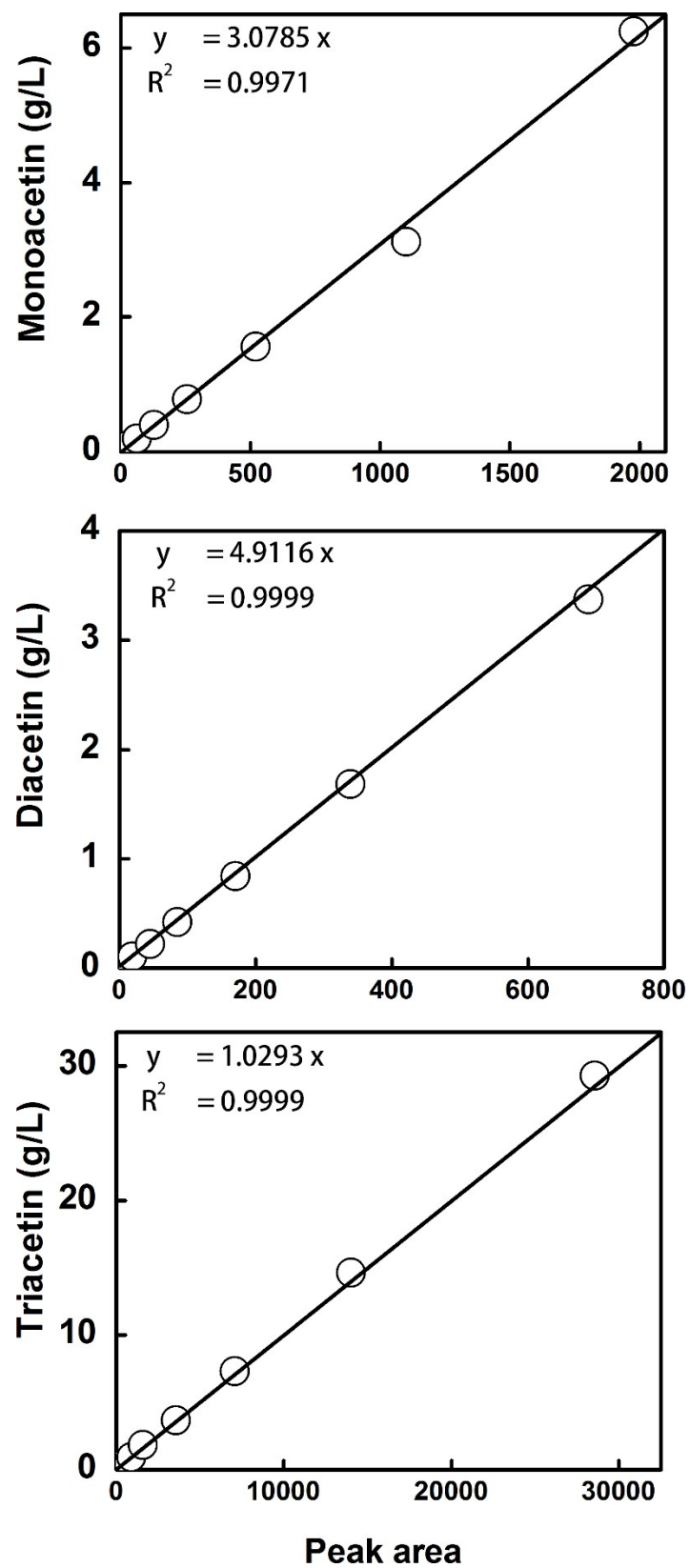


Figure S13. Standard curves of mono-, di- and triacetin.

References

1. J.-H. Kim, C. Wang, H.-J. Jang, M.-S. Cha, J.-E. Park, S.-Y. Jo, E.-S. Choi and S.-W. Kim, *Microbial Cell Factories*, 2016, **15**, 214.