

Additional file

High-yield production of multiple *O*-methylated phenylpropanoids by the engineered *Escherichia coli*-*Streptomyces* cocultivation system

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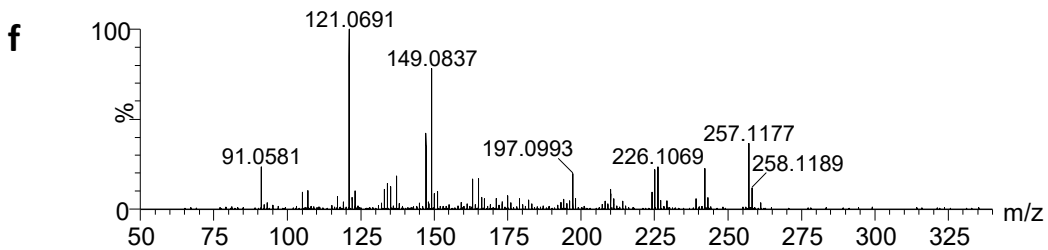
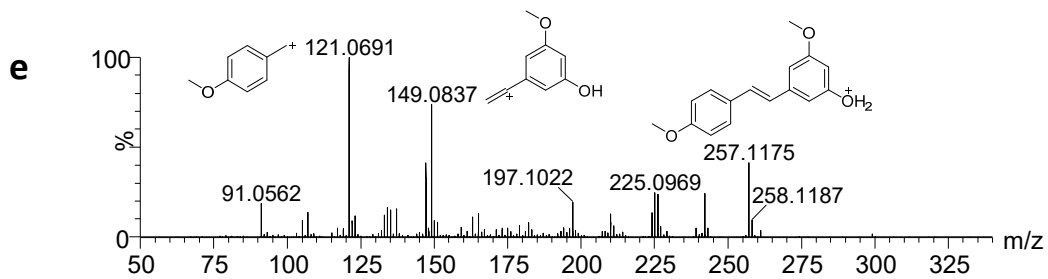
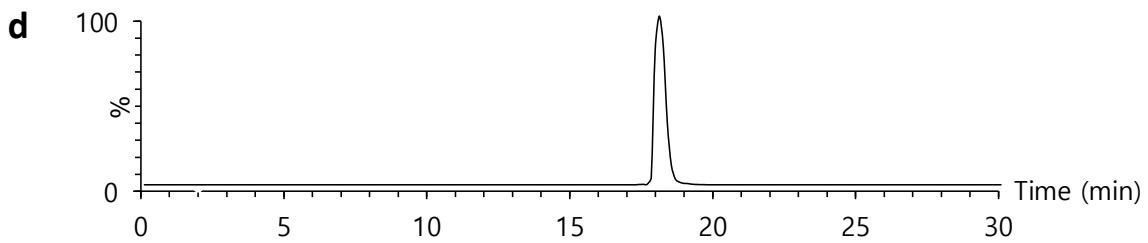
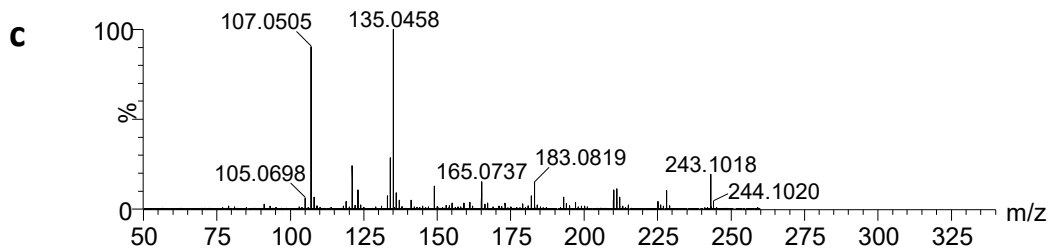
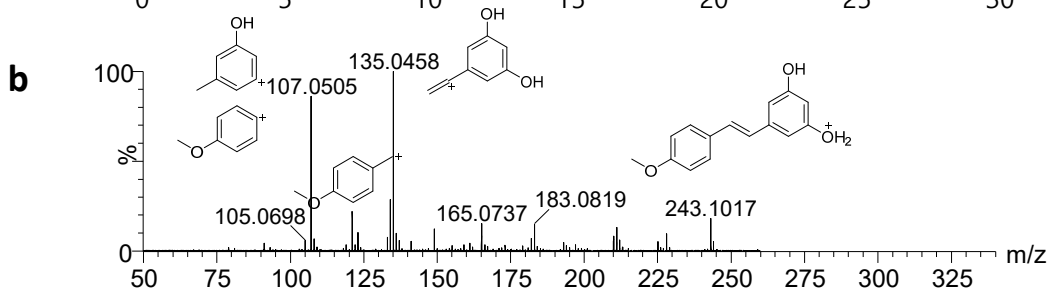
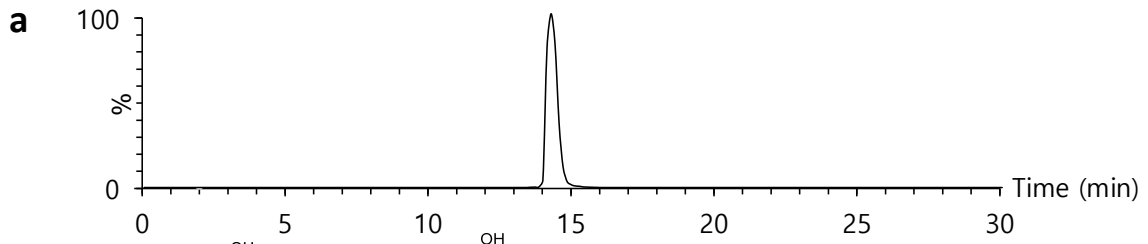
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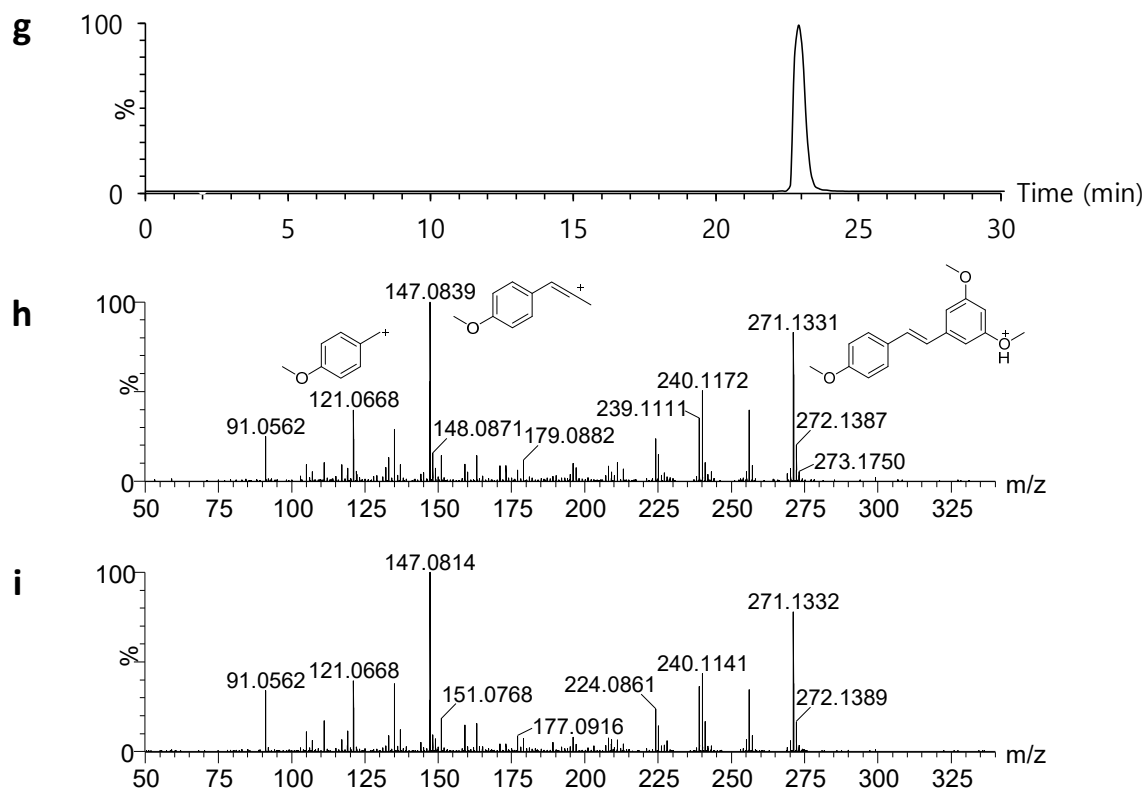
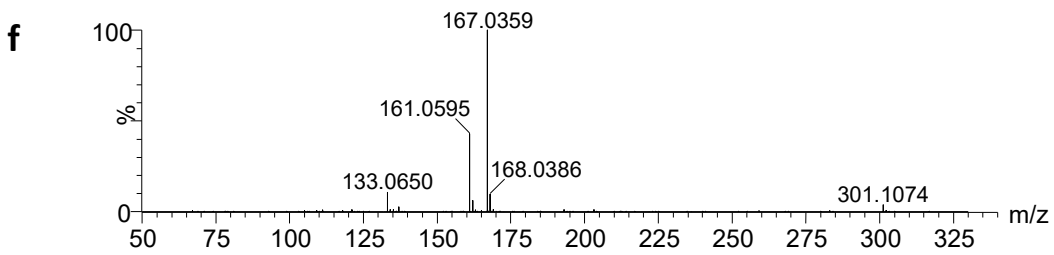
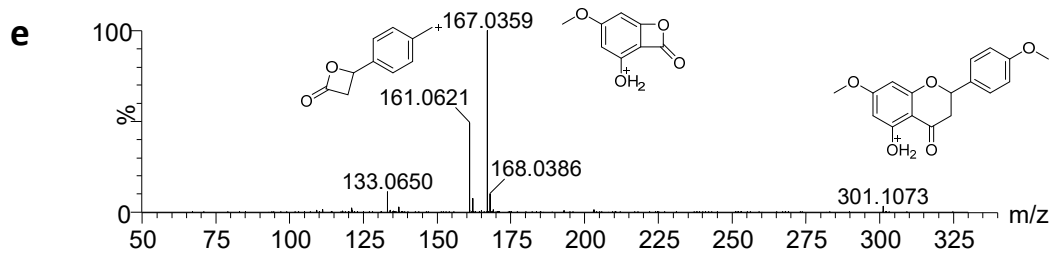
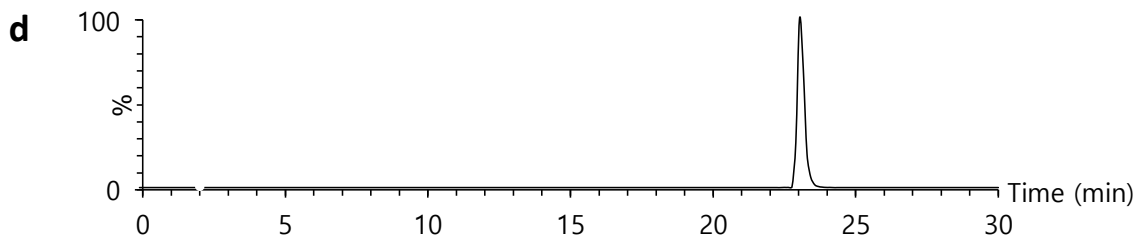
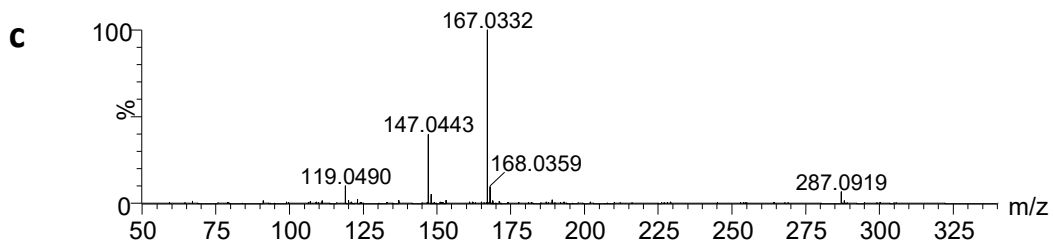
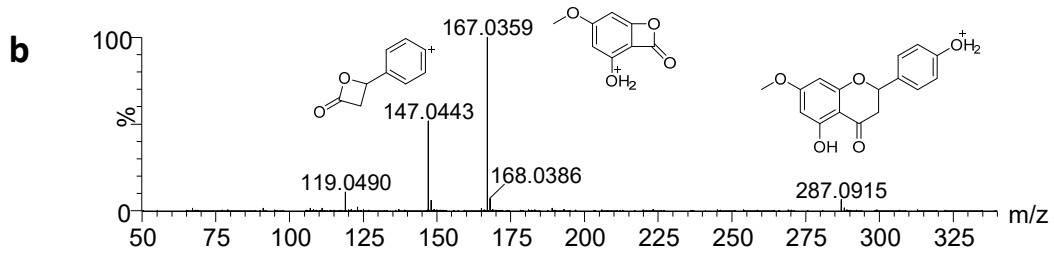
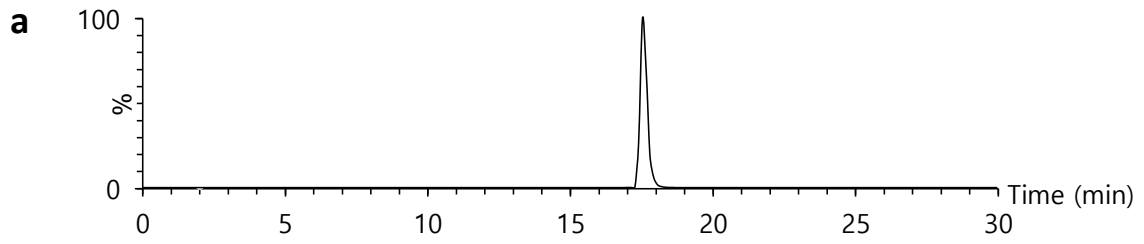


Figure S1. UPLC–qTOF–HR-MS analyses of the methylated stilbenes produced by *S. venezuelae* DHS2001 harboring pSE-SaOMT2 (DHS2001/SaOMT2). (a) UPLC–qTOF–HR-MS chromatogram selected for $m/z=243.1016$ corresponding to standard desoxyrhapontigenin. (b) MS/MS spectrum of standard desoxyrhapontigenin. (c) MS/MS spectrum of desoxyrhapontigenin from the culture extracts of DHS2001/SaOMT2 supplemented with resveratrol. (d) UPLC–qTOF–HR-MS chromatogram selected for $m/z=257.1172$ corresponding to standard 5-hydroxy-3,4'-dimethoxystilbene. (e) MS/MS spectrum of standard 5-hydroxy-3,4'-dimethoxystilbene. (f) MS/MS spectrum of 5-hydroxy-3,4'-dimethoxystilbene from the culture extracts of DHS2001/SaOMT2 supplemented with resveratrol. (g) UPLC–qTOF–HR-MS chromatogram selected for $m/z=271.1329$ corresponding to standard 3,5,4'-trimethoxystilbene. (h) MS/MS spectrum of standard 3,5,4'-trimethoxystilbene. (i) MS/MS spectrum of 3,5,4'-trimethoxystilbene from the culture extracts of DHS2001/SaOMT2 supplemented with resveratrol.



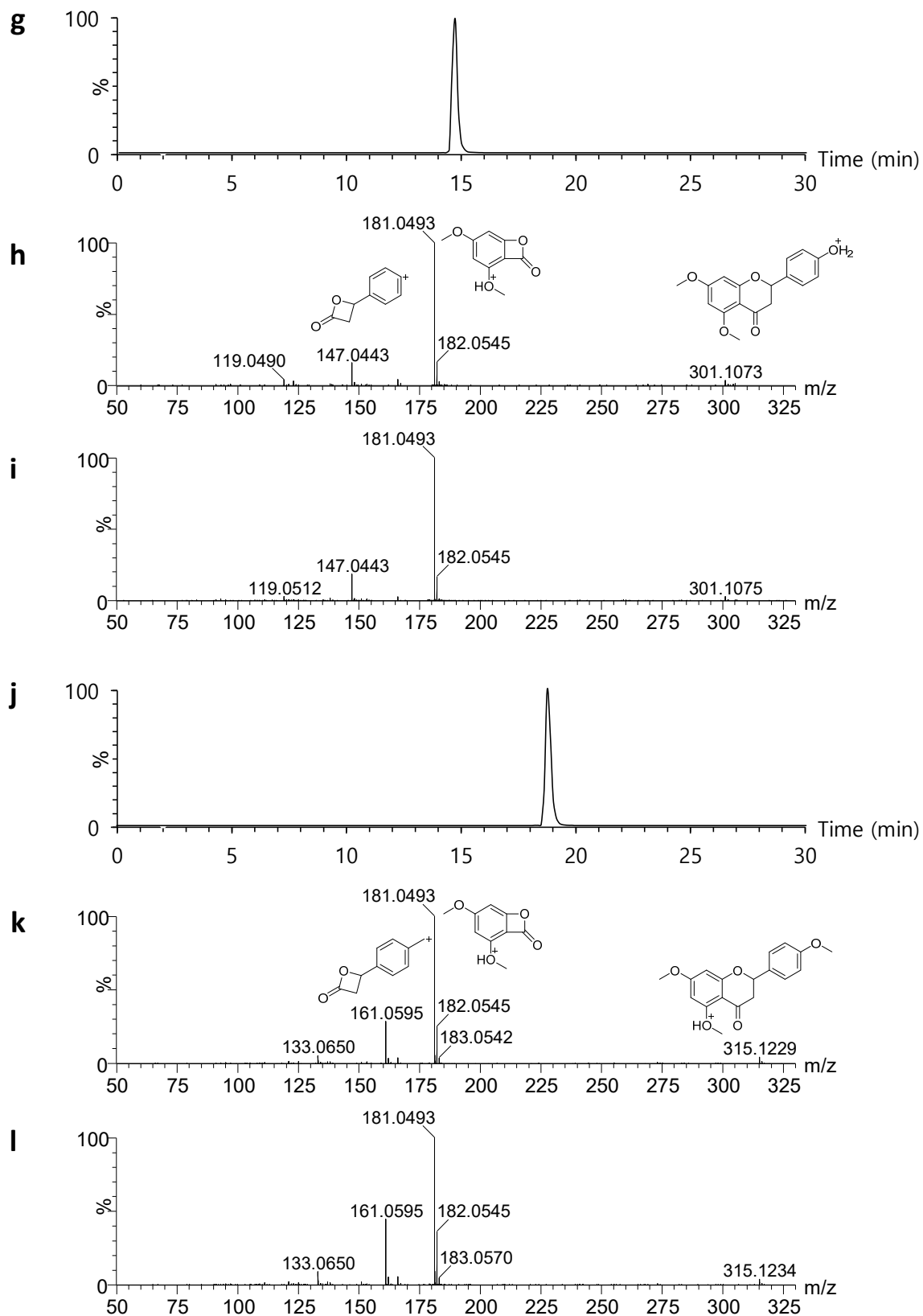
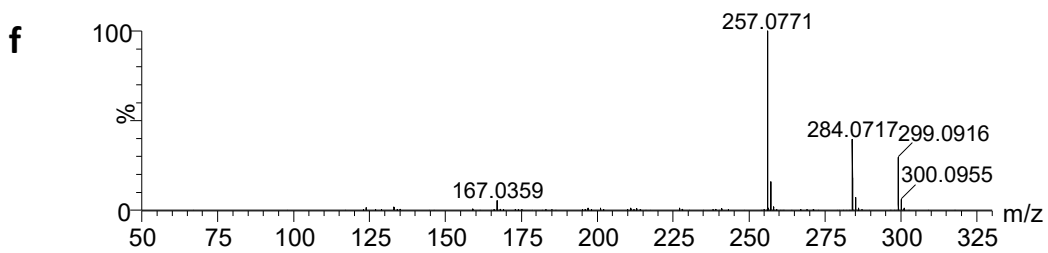
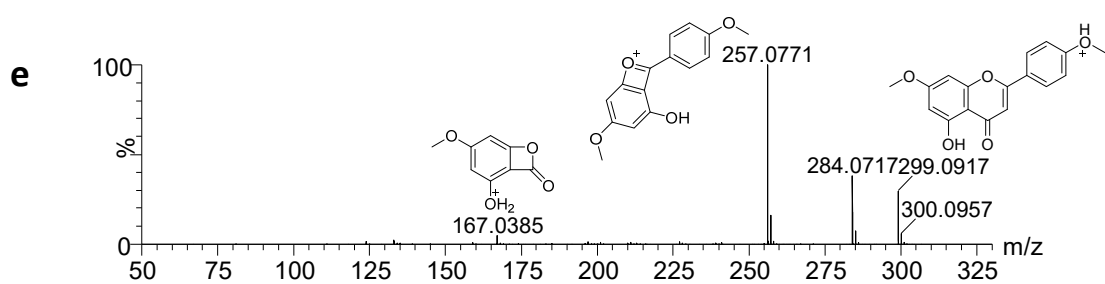
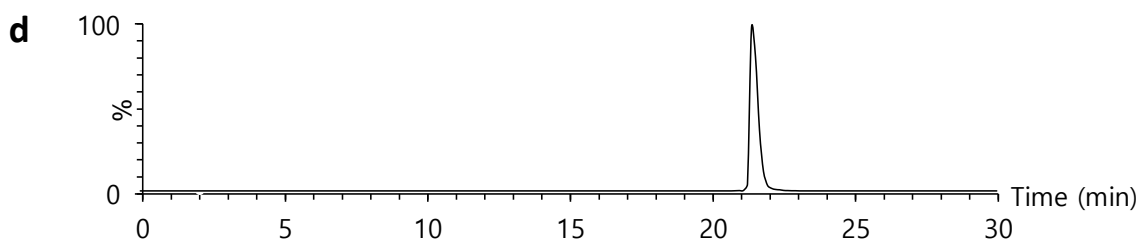
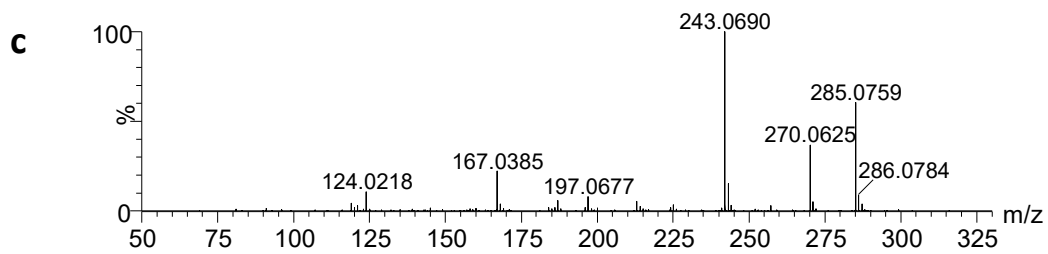
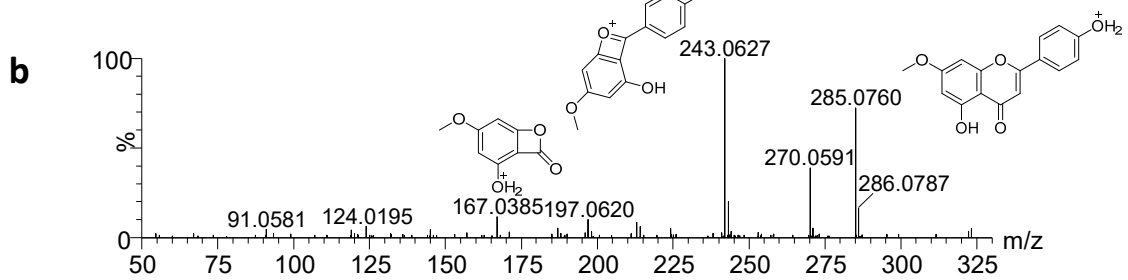
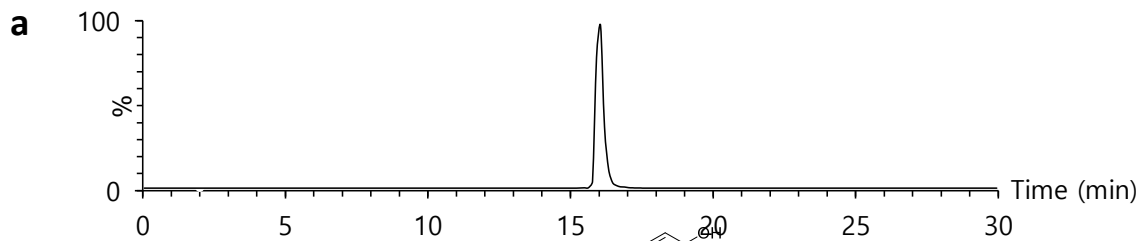


Figure S2. UPLC–qTOF–HR-MS analyses of the methylated flavanones produced by *S. venezuelae*

DHS2001 harboring pSE-SaOMT2 (DHS2001/SaOMT2). **(a)** UPLC-qTOF-MS chromatogram selected for $m/z=287.0914$ corresponding to standard sakuranetin. **(b)** MS/MS spectrum of standard sakuranetin. **(c)** MS/MS spectrum of sakuranetin from the culture extracts of DHS2001/SaOMT2 supplemented with naringenin. **(d)** UPLC-qTOF-HR-MS chromatogram selected for $m/z=301.1071$ corresponding to standard 5-hydroxy-7,4'-dimethoxyflavanone. **(e)** MS/MS spectrum of standard 5-hydroxy-7,4'-dimethoxyflavanone. **(f)** MS/MS spectrum of 5-hydroxy-7,4'-dimethoxyflavanone from the culture extracts of DHS2001/SaOMT2 supplemented with naringenin. **(g)** UPLC-qTOF-HR-MS chromatogram selected for $m/z=301.1071$ corresponding to standard 4'-hydroxy-5,7-dimethoxyflavanone. **(h)** MS/MS spectrum of standard 4'-hydroxy-5,7-dimethoxyflavanone. **(i)** MS/MS spectrum of 4'-hydroxy-5,7-dimethoxyflavanone from the culture extracts of DHS2001/SaOMT2 supplemented with naringenin. **(j)** UPLC-qTOF-HR-MS chromatogram selected for $m/z=315.1227$ corresponding to standard 5,7,4'-trimethoxyflavanone. **(k)** MS/MS spectrum of standard 5,7,4'-trimethoxyflavanone. **(l)** MS/MS spectrum of 5,7,4'-trimethoxyflavanone from the culture extracts of DHS2001/SaOMT2 supplemented with naringenin.



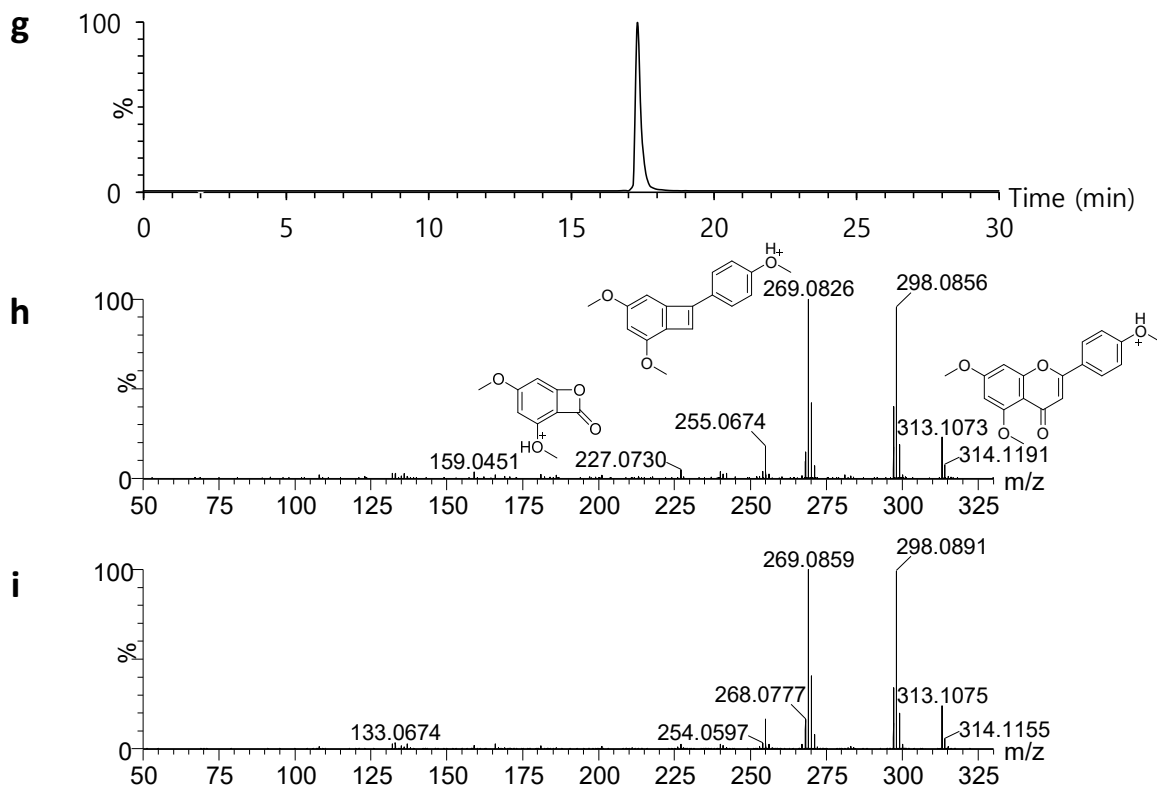
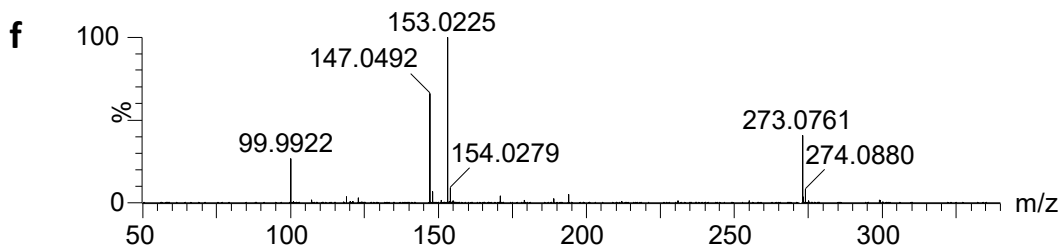
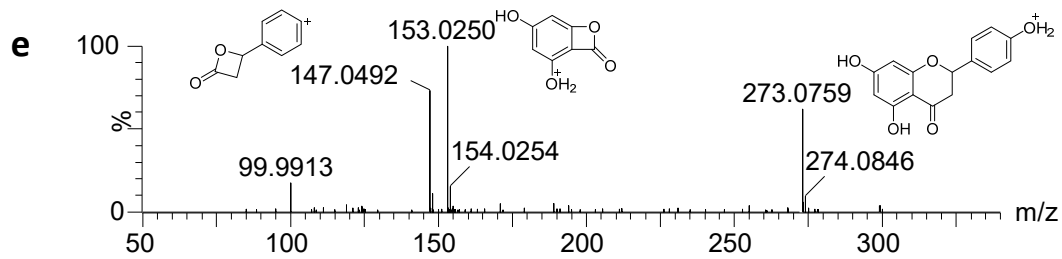
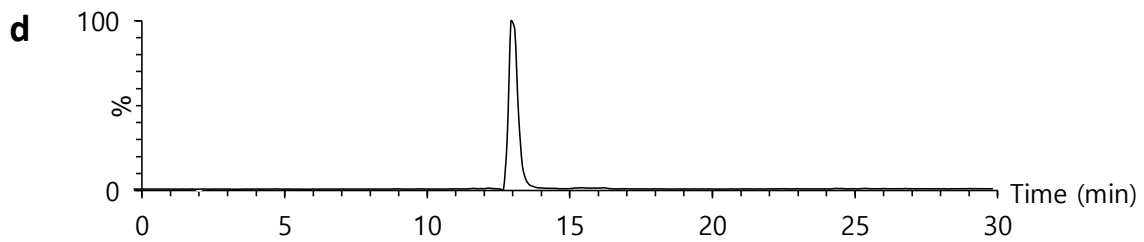
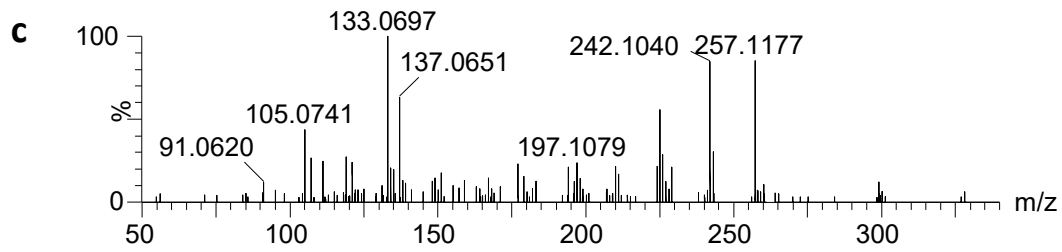
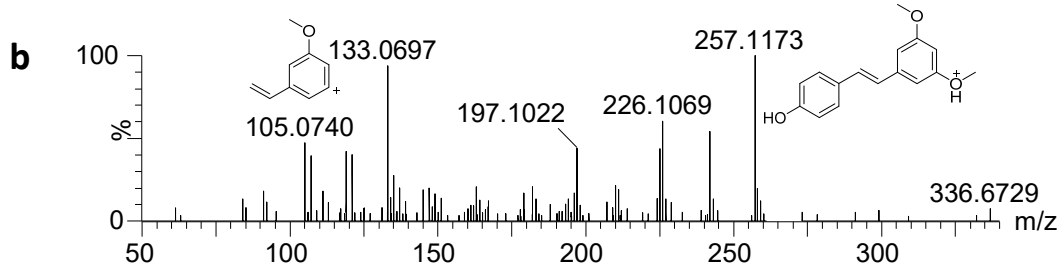
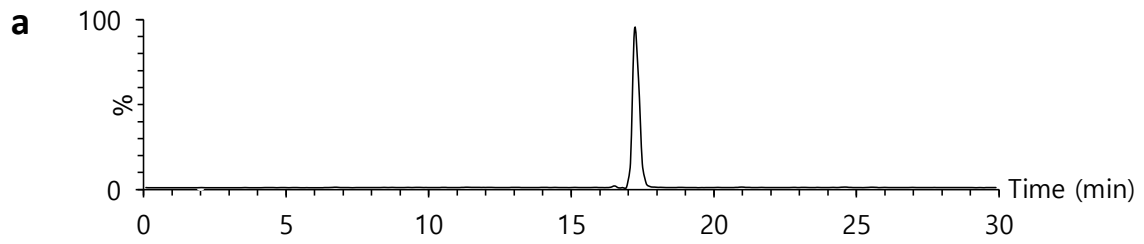


Figure S3. UPLC–qTOF–HR-MS analyses of the methylated flavones produced by *S. venezuelae* DHS2001 harboring pSE-SaOMT2 (DHS2001/SaOMT2). (a) UPLC–qTOF–HR-MS chromatogram selected for $m/z=285.0757$ corresponding to standard genkwanin. (b) MS/MS spectrum of standard genkwanin. (c) MS/MS spectrum of genkwanin from the culture extracts of DHS2001/SaOMT2 supplemented with apigenin. (d) UPLC–qTOF–HR-MS chromatogram selected for $m/z=299.0914$ corresponding to standard 5-hydroxy-7,4'-dimethoxyflavone. (e) MS/MS spectrum of standard 5-hydroxy-7,4'-dimethoxyflavone. (f) MS/MS spectrum of 5-hydroxy-7,4'-dimethoxyflavone from the culture extracts of DHS2001/SaOMT2 supplemented with apigenin. (g) UPLC–qTOF–HR-MS chromatogram selected for $m/z=313.1071$ corresponding to standard 5,7,4'-trimethoxyflavone. (h) MS/MS spectrum of standard 5,7,4'-trimethoxyflavone. (i) MS/MS spectrum of 5,7,4'-trimethoxyflavone from the culture extracts of DHS2001/SaOMT2 supplemented with apigenin.



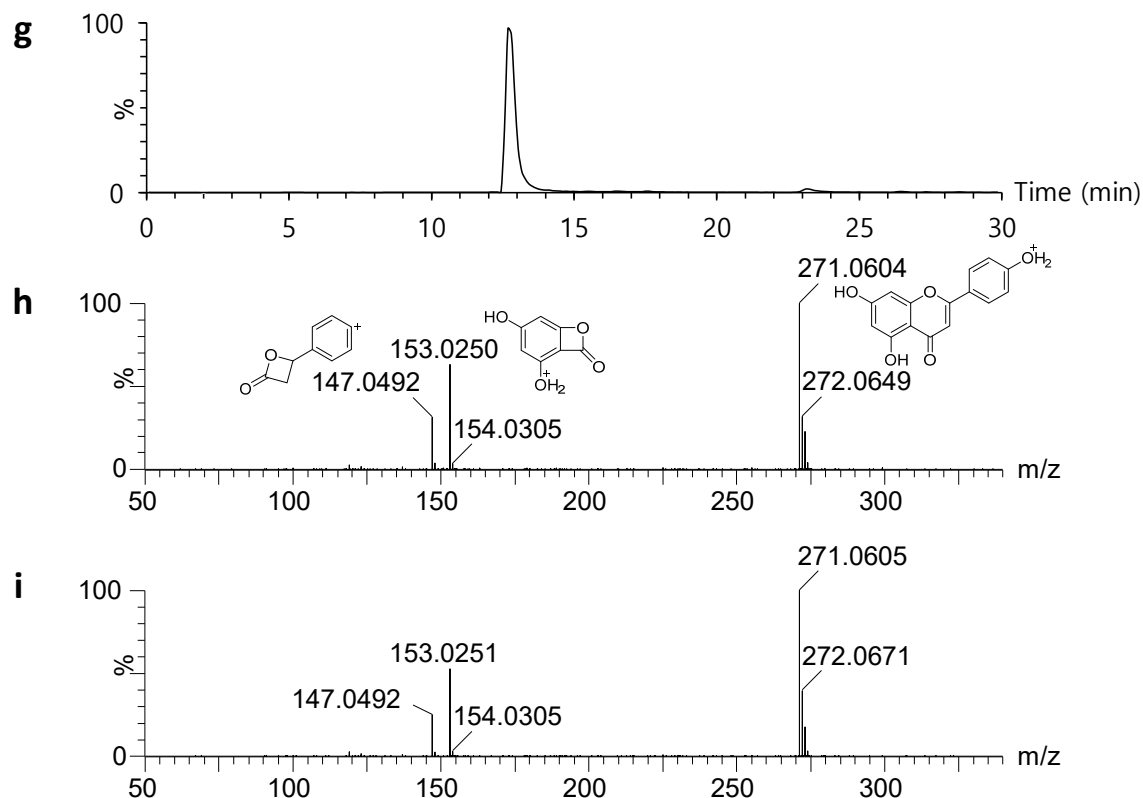


Figure S4. UPLC–qTOF–HR-MS analyses of the pterostilbene, naringenin and apigenin produced by *E. coli* mutants. **(a)** UPLC–qTOF–HR-MS chromatogram selected for $m/z=257.1172$ corresponding to standard pterostilbene. **(b)** MS/MS spectrum of standard pterostilbene. **(c)** MS/MS spectrum of pterostilbene obtained from BL21/PTS supplemented with 4-coumaric acid. **(d)** UPLC–qTOF–HR-MS chromatogram selected for $m/z=273.0757$ corresponding to standard naringenin. **(e)** MS/MS spectrum of standard naringenin. **(f)** MS/MS spectrum of naringenin obtained from BL21/NRG supplemented with 4-coumaric acid. **(g)** UPLC–qTOF–HR-MS chromatogram selected for $m/z=271.0601$ corresponding to standard apigenin. **(h)** MS/MS spectrum of standard apigenin. **(i)** MS/MS spectrum of apigenin obtained from BL21/APG supplemented with 4-coumaric acid.

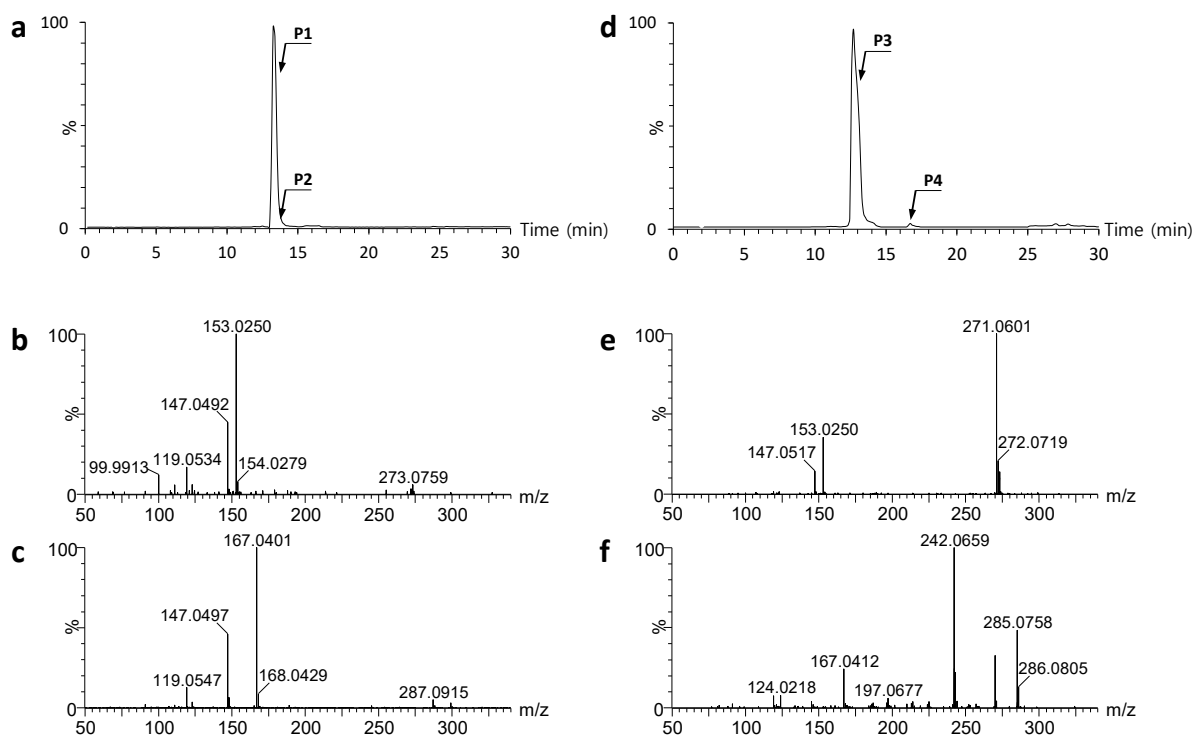


Figure S5. UPLC–qTOF–HR-MS analyses of methylated flavanone and methylated flavone produced by *E. coli* BL21(DE3) harboring pNRG-SaOMT2 and pAPG-SaOMT2, respectively. (a) UPLC–qTOF–HR-MS chromatogram selected for $m/z=273.0757$ and 287.0914 corresponding to naringenin (**P1**) and sakuranetin (**P2**), respectively, of culture extracts from BL21/NRG-SaOMT2. (b) MS/MS spectrum selected for $m/z=273.0757$ of naringenin obtained from BL21/NPG-SaOMT2 supplemented with 4-coumaric acid. (c) MS/MS spectrum selected for $m/z=287.0914$ of sakuranetin obtained from BL21/NPG-SaOMT2 supplemented with 4-coumaric acid. (d) UPLC–qTOF–HR-MS chromatogram selected for $m/z=271.0601$ and 285.0757 corresponding to apigenin (**P3**) and genkwainin (**P4**), respectively, of culture extracts obtained from BL21/APG-SaOMT2. (e) MS/MS spectrum selected for $m/z=271.0601$ of apigenin obtained from BL21/APG-SaOMT2 supplemented with 4-coumaric acid. (f) MS/MS spectrum selected for $m/z=285.0757$ of genkwainin obtained from BL21/APG-SaOMT2 supplemented with 4-coumaric acid.

Table S1. List of oligonucleotide primers used in this study

Primers	Primer sequences (5' to 3', restriction site underlined)
SaOMT2-1_F	<u>GAATTC</u> ATGAGCTGCCGCACCGGC
SaOMT2-1_R	<u>CCTAGG</u> TCAACCTACGGCCCGCAA
SaOMT2-H1_R	<u>CCTAGG</u> TCAGTGGTGGTGGTGGTGGTGGCCAACCGCGCGCAGTTC
SaOMT2-H2_F	<u>TCTAGAA</u> AATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGAGTTGT CGTACTGGT
SaOMT2-H2_R	<u>GCGGCCG</u> CACCTACGGCCCGCAA
Os4CL_F	<u>TCTAGAG</u> GAGGATTACAATGGGGTCGGTGGCGGCG
Os4CL_R	<u>AAGCTT</u> TTAGCTGCTTTTGGGCGC
VvSTS_F	<u>AAGCTT</u> AGGAGGATTACAAAATGGCTTCAGTCGAGGAA
VvSTS_R	<u>CCTAGG</u> TTAATTTGTAACCAT
VvROMT_F	<u>CCTAGG</u> GAGGAGGATTACAAAATGGATTTGGCAAACGGT
VvROMT_R	<u>GCGGCCG</u> CTCAAGGATAAACCTCAAT
Os4CL-2_F	<u>AAGCTT</u> AGGAGGATTACAATGGGGTCGGTGGCGGCG
Os4CL-2_R	<u>GCGGCCG</u> CTTAGCTGCTTTTGGGCGC
PeCHS_F	<u>CCATGG</u> AGATGGCACCGTCGATTGAG
PeCHS_R	<u>AAGCTT</u> AATAAGCTTTCATGAGTAAATTGTTTG
MtCHI_F	<u>CATATG</u> ATGGCTGCATCAATCACC
MtCHI_R	<u>CCTAGG</u> GGTACCTCAGTTTCCAATCTTGAA
PcFNS_F	<u>GGTACC</u> AGGAGGATTACAATGGCTCCTACAACAATA
PcFNS_R	<u>CCTAGG</u> CTAAGCTAAATTTTCATC
SaOMT2-2_F	<u>CCTAGG</u> GAGGAGGTCTCGCATGAGTTGTCGTAAGTGGT
SaOMT2-2_R	<u>GCGGCCG</u> CTCAACCTACGGCCCGCAA