# Supporting information for:

**Efficient biosynthesis of salidroside via artificial *in vivo* enhanced UDP-glucose system using cheap sucrose as substrate in *Saccharomyces cerevisiae***

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**Supplementary Table S1. *S*trains and plasmids used in this study**

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| --- | --- | --- |
| Strains and plasmids | Relevant genotype or description | Source or reference |
| *Strains* |  |  |
| QH01 | *S. cerevisiae* BY4743 integrating PcDHPAAS | This study |
| QH02 | QH01 harboring RrUGT33-pTEF426 | This study |
| QH03 | QH01 harboring PZW0320 | This study |
| QH04 | BL21(DE3) ∆pheA | This study |
| QH03-hxt-null | *hxt1Δ::HIS3::Δhxt4 hxt2Δ::HIS3 hxt3Δ::LEU2::hxt6 hxt5::LEU2*  *hxt7::HIS3 gal2Δ derivative of S. cerevisiae* BY4743 | This study |
| DWL01 | BL21(DE3) harboring AADW01 | This study |
| DWL02 | BL21(DE3) harboring AADW02 | This study |
| DWL03 | BL21(DE3) harboring AADW03 | This study |
| DWL04 | BL21(DE3) harboring AADW04 | This study |
| *Plasmids* |  |  |
| GmSUS-pTEF426 | pTEF426 carries GmSUS, AmpR | This study |
| RrUGT33-pTEF426 | pTEF426 carries RrUGT33, AmpR | This study |
| PDWZ0320 | ptDH3-GmSUS-ptDH3-RrUGT33-pTEF426 carrying GmSUS and RrUGT33, AmpR | This study |
| AADW01 | pET28a carries an acetaldehyde synthases gene encoded for PcDHPAAS from Psilocybe cubensis, AmpR | This study |
| AADW02 | pET28a carries an acetaldehyde synthases gene from *Rhodiola* gene encoded for Rr4HPAAS, AmpR | This study |
| AADW03 | pET28a carries a paralogou tyrosine decarboxylase gene encoded for PsTyDC9 from *Olea europaea*, AmpR | This study |
| AADW04 | pET28a carries a tyrosine decarboxylase gene encoded for TDCs from *Olea europaea*, AmpR | This study |

**Supplementary Table S2. Nucleotide sequences of codon optimized genes**

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| Genes | Direction | Sequence |
| *GmSUS* | Forward | GCATAGCAATCTAATCTAAGATGGCTACTGACAGATTGACC |
| *GmSUS* | Reverse | GCGTGACATAACTAATTACATGATCATTCAGCGGCTAAAGGG |
| *PcHPAAS* | Forward | GAAAACTTGTACTTCCAGGCCCATGGCATGGGCTCCATCGATAATC |
| *PcHPAAS* | Reverse | CTCGAATTCGGATCCGCCATGG TTAGGATAAAATATTCACGATCTTCT |
| *RrUGT33* | Forward | GCATAGCATAATCTAAGTCTAGAACTAGTATGGCATGAGCTTAATTGAAAAACCACTCACG |
| *RrUGT33* | Reverse | CTCGAATTCGGATCCGCCATGGCTAACGGATATGTTTTGTTTTTGAGAGCAGGAC |

**Supplementary Table S3. Nucleotide sequences of codon optimized genes**

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| --- | --- |
| GmSUS | ATGGCTACTGACAGATTGACCAGAGTCCACAGTTTGAGAGAAAGATTGGATGAAACCTTGACCGCTAACAGAAACGAAATCTTGGCCCTGCTATCTAGAATTGAAGCTAAGGGTAAGGGTATTTTGCAACACCACCAAGTCATTGCTGAATTTGAAGAAATCCCAGAAGAAAACAGACAAAAGCTCACTGATGGTGCTTTCGGTGAAGTTTTGAGATCTACCCAAGAAGCTATCGTTTTGCCTCCATGGGTCGCTTTGGCTGTTAGACCAAGACCAGGTGTTTGGGAGTACTTGCGTGTTAACGTCCATGCTTTAGTCGTTGAAGAATTGCAACCAGCTGAATACTTACATTTCAAGGAAGAATTGGTTGACGGTTCCTCTAACGGAAACTTCGTCTTGGAGTTGGACTTCGAACCATTCAACGCTGCTTTCCCAAGACCAACCTTGAACAAGTCCATCGGTAACGGTGTCCAATTTTTGAACCGTCACTTGTCTGCTAAGTTATTCCACGACAAAGAGTCTTTGCACCCATTGCTAGAATTCTTGAGATTGCATTCTGTTAAGGGTAAGACTTTGATGTTGAACGACAGAATCCAAAACCCAGATGCTTTGCAACACGTCTTAAGAAAGGCCGAAGAATATTTGGGTACTGTTCCACCAGAAACCCCTTACTCTGAATTCGAACACAAATTCCAAGAAATTGGTTTAGAAAGAGGTTGGGGTGACAACGCCGAAAGAGTTTTAGAATCTATTCAATTGTTACTTGATTTATTGGAAGCTCCAGATCCATGTACCCTGGAAACTTTCTTGGGTAGAATTCCAATGGTTTTCAACGTTGTCATCTTGTCCCCTCACGGTTACTTTGCTCAAGATAACGTTTTGGGTTACCCTGACACTGGTGGTCAAGTCGTCTACATTTTGGACCAAGTACGTGCTTTGGAAAACGAAATGTTGCACAGAATCAAGCAACAAGGTTTGGACATCGTCCCACGCATTTTGATTATTACTCGTTTGCTTCCAGATGCTGTTGGTACCACCTGTGGCCAAAGACTTGAAAAGGTTTTCGGTACTGAACATTCCCACATCTTGAGAGTTCCATTCAGAACTGAAAAGGGTATTGTTAGAAAGTGGATCTCTAGATTCGAAGTCTGGCCATACTTGGAAACTTACACTGAAGATGTCGCTCACGAACTCGCAAAGGAATTACAAGGTAAACCAGACTTGATTGTCGGTAACTACTCTGACGGTAACATCGTTGCTTCTTTGTTGGCTCACAAGTTAGGTGTCACTCAATGTACCATCGCCCATGCCTTGGAAAAGACCAAGTACCCAGAATCCGACATTTACTGGAAGAAGTTAGAAGAACGTTACCACTTCTCTTGCCAATTCACTGCTGATTTGTTCGCTATGAACCACACCGACTTCATCATCACTTCTACTTTCCAAGAAATTGCTGGTTCTAAGGATACCGTCGGTCAATACGAATCTCACACTGCTTTTACTTTGCCAGGTTTATACAGAGTTGTTCACGGTATCGATGTTTTTGACCCAAAGTTCAACATTGTATCTCCAGGTGCTGACCAAACCATCTACTTCCCACACACTGAAACATCCAGACGGTTAACCTCTTTCCATCCAGAAATTGAAGAATTGCTTTATTCCTCAGTTGAAAATGAAGAACACATCTGTGTCTTGAAGGACAGATCCAAGCCAATTATCTTCACCATGGCTAGATTAGACCGAGTTAAGAACATCACTGGCTTGGTCGAATGGTACGGTAAGAACGCTAAGTTGCGTGAATTAGTCAACCTGGTTGTTGTTGCCGGTGATAGAAGAAAGGAATCAAAGGACTTGGAAGAAAAGGCTGAAATGAAGAAAATGTACGGTTTGATCGAAACCTACAAGTTGAATGGTCAATTCAGATGGATTTCTTCTCAAATGAACCGTGTCAGAAATGGTGAATTGTACAGAGTCATCTGTGATACTAGAGGTGCCTTCGTTCAACCAGCCGTCTACGAAGCTTTCGGTTTGACCGTTGTTGAAGCGATGACTTGTGGTCTGCCAACTTTTGCCACATGTAACGGTGGTCCAGCTGAAATCATCGTCCACGGCAAGTCTGGTTTCCACATTGACCCATACCATGGTGATAGAGCTGCTGATTTGTTGGTCGATTTCTTTGAAAAGTGTAAGTTGGATCCGACACACTGGGACAAGATCTCCAAGGCTGGTTTGCAAAGAATCGAAGAAAAATACACCTGGCAAATATACTCCCAACGTTTGTTGACCTTGACCGGTGTTTATGGTTTCTGGAAGCACGTTTCCAACTTGGACAGAAGAGAATCCAGAAGATACTTGGAAATGTTCTACGCTCTAAAATATAGAAAATTGGCCGAATCCGTCCCTTTAGCCGCTGAATGA |

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| RrUGT33 | ATGAGCTTAATTGAAAAACCACTCACGGCCATAGAGACTCGTGAAAAACCACACGCTGTGTGCATCCCATACCCAGCTCAAGGCCATATCAATCCCATGATGCAACTTGCAAAGCTCCTCCACCACTCTGGTTTCCACATAACGTTTGTCCACACTGAGTATAATTATGACCGTCTAGTGAAGTCTCAAGGTTCAGCTTGTGTGGCTGGTTTACCGGATTTCCGCTTTGAAGCCATCCCAGATGGCTTGCCCTCGACGAATGGTGATGTTACTCAAGACATTCCTCTGTTGAGTAGCTCTACTTCTAAAACCTGCTTGAAGCCGTTTAAGGAGTTATTGAAGAGGTTGCAGGACAAATGCAAAGAGTTACCTGATGATGTTCCGCCTCTGTCGTGCATCGTGTCTGATGCAGCCATGTCGTTTACGATCGATGCATCTGAGGAGTTTGGAGTGCCCATAGCGCTTCTTTGGACTGCAAGTGCCTGCGGGTTCTTGGGTTACACGCATTACCCATATCTAATTGACAGAGGTGTCATCCCATTGAAAGATGAGAGCCAATTAACAAACGGATACCTAGATATGAGCATAGATGGCATACCTTGTATGGAAGGTATCCGCTTACGAGACCTCCCAAGCTTTCTACGCACAACTGATTTAGATGATATGATGTTTAGTTATATACTGCACGAAATAAAACAAGTTTCAAGAGGCAGTGCTATCATTCTGAACACCTTTGAAGCTTTGGACCATGATGTCTTGGATAGTCTCTCCAAAATTTACCAAAATGTCATCCTGCCAGTTGGCCCTCTACATGTCTCGCTCAACAAGATCCCAAAACACTACCCACTTCAATCTTTAAGCTCGAATTTATGGAAAGATGACACAGACTGCATTCCCTGGCTGAGCTCTAAGGCTTCAAAATCAGTTATATACGTTAACTTTGGGAGCATCACGACGGTATCACCAAAACAAATTGTGGAGTTTGCGTGGGGATTGGCTAACAGCAAACACCCTTTCCTTTGGATAATCAGACCGGACTTGGTGGCAGGTGAGGCATCCATCATTCCGCAGGACTTCATGGATGAAACAAAAGGAAGAGGTTTGTTGGCTGGTTGGTGTGACCAAGAGCTTGTTCTCAACCATCCATCCATTGGAGGGTTTCTTACGCACTGTGGCTGGAACTCAATTATTGAAAGCATTAGCGCAGGAGTCCCTACGGTCTGCTGGCCATTTTTTGCTGAGCAGCAAACAAATTGTTGGTTTGCTTGCAAAAAATGGTGCATTGGGATGGAGATGCATACTGATGTAAAGAGGGATGAGGTTGACAAGCTGTTGAGAGAGCTAATGGAAGGTGACAAAGGGGAGGAGTTGAAGAGGAAGGCAACCAACTGGAAGAGGCTGGCAGAAGAAGCTGTTTCCTCCACTGGCTTATCAACCTTAAACTTCAGGACGTTAGTGAATCAAGTCCTGCTCTCAAAAACAAAACATATCCGTTAG |

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| PcDHPAAS | ATGGATATCGAACAATTTAGGAAAGCAGGCTATCAAGCCATTGATCGCATATGCGATTACTACTATTCTTTGCAAAATTCAACTGTTATGTCTAAGGTTGAGCCTGGATATTTAAGGCAGCACATTCCTTTGGAAGCACCCGAAGAGGGTGAAGACTTTCAAATCATTGCAGACGATTACCAGAAATTTATCGTACCAGGTTTAACGCATTGGCAGCATCCTTCTTTCTTTGCCTACTTCCCTACGGCATGCACATTCGAGGGGATTTTGGGAGATTTGTATGCATCAAGCACTTGCAACCCTGGTTTCAATTGGTTGGCGAGTCCTGCTTGCACAGAGTTAGAGGCAATTGTTATGGATTGGGCCGCCAATCTCCTTGGGTTATCATCAGCCTTCAAGAATTCCTCAGGAATTGGCGGTGGGGCAATTCAAACAACAGCGTCAGACTCAGTCCTCATTACGGTTGTCGCCGCTCGATCCATGTACCAACGTAACCATCCAGATGTAAAAATGGAGGATCTCGTTATATACACAACTACACAAACACATTCTCTGGGCGCCAAAGCCGGTATCGTTCTTGGACTACAAGTTCGCTCGATCGAGGTCCTCGCAGAAGAAAAATACGCTTTGAGAGGTCAAGCCCTTCGTGACGCGTTAGAAGAAGACCGAAAACTAGGACGCAAGCCTTTCATATTGATTGCCACCGTCGGATCGACTTCTTCAGGAGCTGTGGACAACTTAATGGAAATCCATCAAATATCAAAGGAACAACCTGACCTATGGGTTCATGTTGACGCTGCTTGGGCTGGCGTTGCCCTGTCCTGCCCGGAAACTCGCAAAAATTTATATCTAGAGGATATAAATGCCTTTGTTCATTCGTTCTGTACCAACTTCCACAAGTGGGGACTTGTTAATTTTGACTGCTCTGCCCTTTGGGTCCGAGATCGCAAATATCTTACTGATGCTTTGGACATCACTCCTGCCTTCTTACGGACAAAACAAGGAGATGCTGGCACAGTTATTGACTACCGAAACTGGCATTTGGGGTTGGGTCGACGATTCCGTTCCTTAAAAATGTGGTTTGTTTTGAGAGGATTTGGCGCAGAGGGTTTCCGCATGTACATCCGACGATGCATAGATCTGAACCAGAAATTCGCACAACTCGTTCGCGATTCTGAGGAGTTGTCTTTGGTTACCGACCCCTCTCTGGCCCTGACAGTATTTCGAGTTGTGCCCAAGTTGCAATCTGAAGATCAACCACCGCTTTCTACAGAAACCCTGAATGAAATCAACAGCATCTTCTACGGCCGCGTGTCCTCGCGAAGCGACATCATGCTCACTCAAACCAACTTGAACGGCATATTCTGCATCCGGCTTGCAGTCGGGGCAGCCAGAACAACAGAACAGCATATCCAGGATGCATTTACCATTATCGAGAAAGAGGCGAAGGCTGCTATTGAAGCTTGGAAAACTATCAACGGAACTATCACTGAATA |

**Supplementary Table S4** Comparison of cost with different strains. Strain QH02 was fermented with 20 g/L glucose and 2 g/L L-tyrosine for 96 h to obtain the cost of 1 kg salidroside for comparison. The QH03-hxt-null strain was incubated with 20 g/L glucose and 2 g/L L-tyrosine for 96 h. The QH03-hxt-null and QH04 co-culture systems were fermented with 15 g/L sucrose and 5 g/L glucose as substrates for 96 h. Note: Cost was calculated at shaker level.

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| Strains | Substrates | Cost (dollar per kilogram salidroside) | L-tyrosine  utilization (%) | Salidroside titer (g/L) | Cost saving (%) |
| QH02 | Glucose, L-tyrosine | 161 | 43% | 1.42 | 0 |
| QH03-hxt-null | Sucrose, L-tyrosine | 73 | 95% | 3.14 | 54 |
| QH03-hxt-null and QH04 | Sucrose, Glucose | 30 | - | 3.8 | 81 |

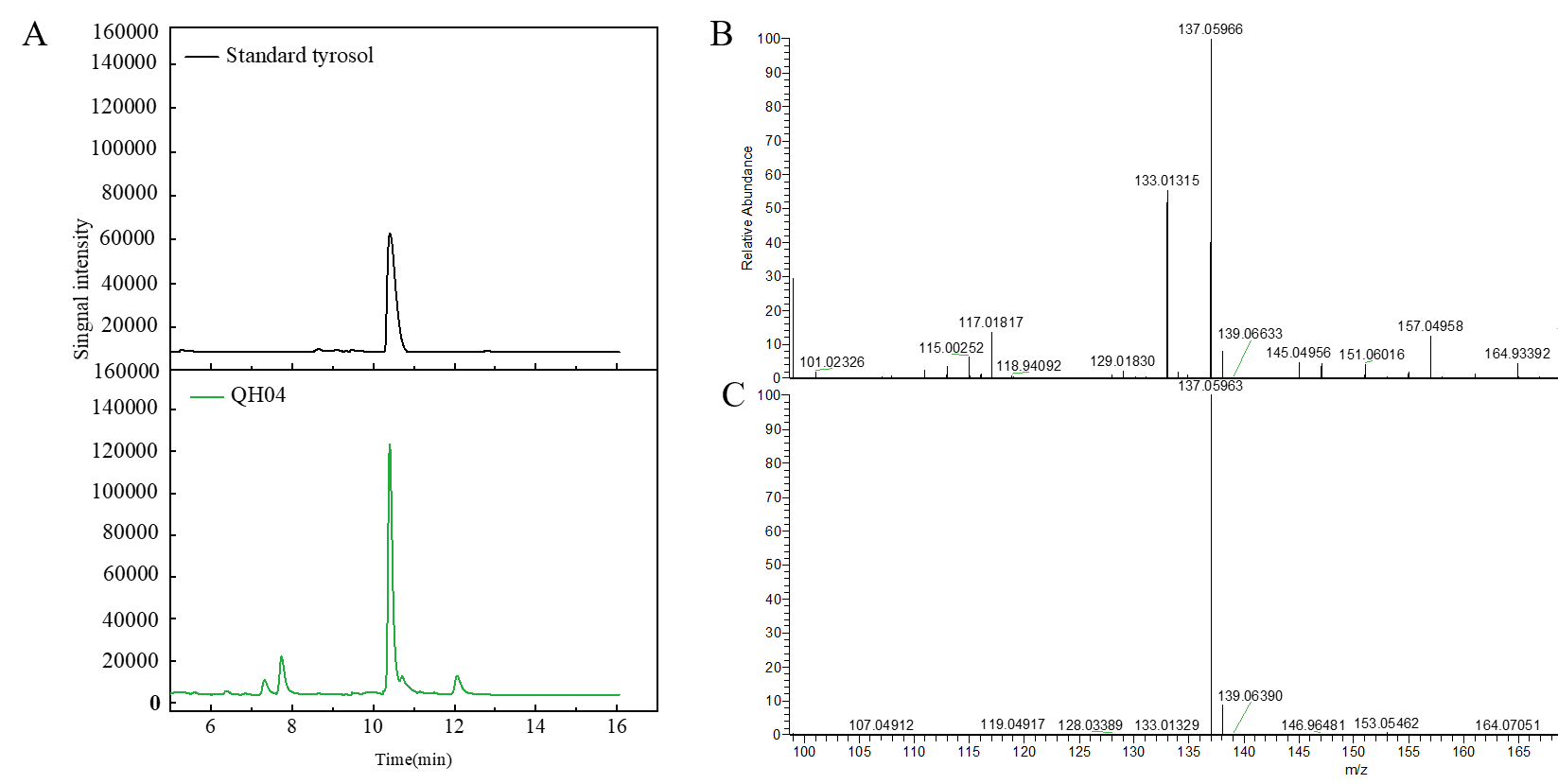


Figure. S1 A. HPLC analysis of fermentation broth of QH04 strains. B. The LC–MS/MS of the fermentation supernatant sample of strain QH04. C. The LC–MS/MS of the standard tyrosol. Strain QH04 produced a major product that has identical retention time and yields identical ([M]-) ions to standard tyrosol and the primary ion fragment at m/z 137([M]-) corresponds to tyrosol with molecular weight of 138.

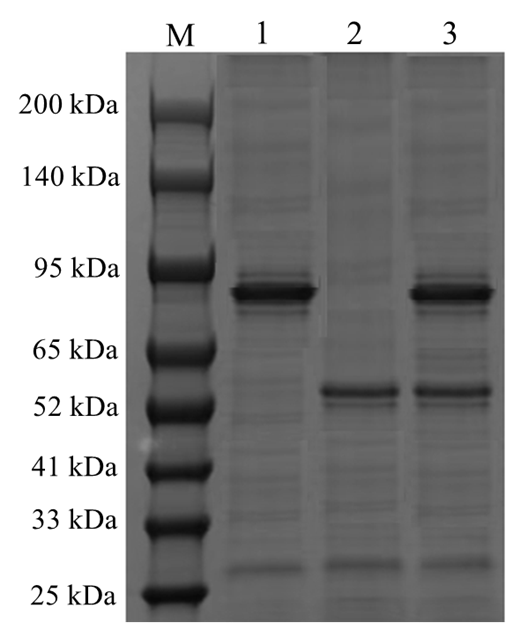


Figure. S2 Co-expression of *RrUGT33* genes and *GmSUS* in *S. cerevisiae*. Analysis of GmSUS and the proteins obtained from crude extraction GmSUS and RrUGT33 co-expressing *S. cerevisiae* cells by coomassie-stained polyacrylamide gel. M: Marker, (1) GmSUS singly expressed; (2) RrUGT33 singly expressed; (3) RrUGT33 co-expressed with GmSUS.

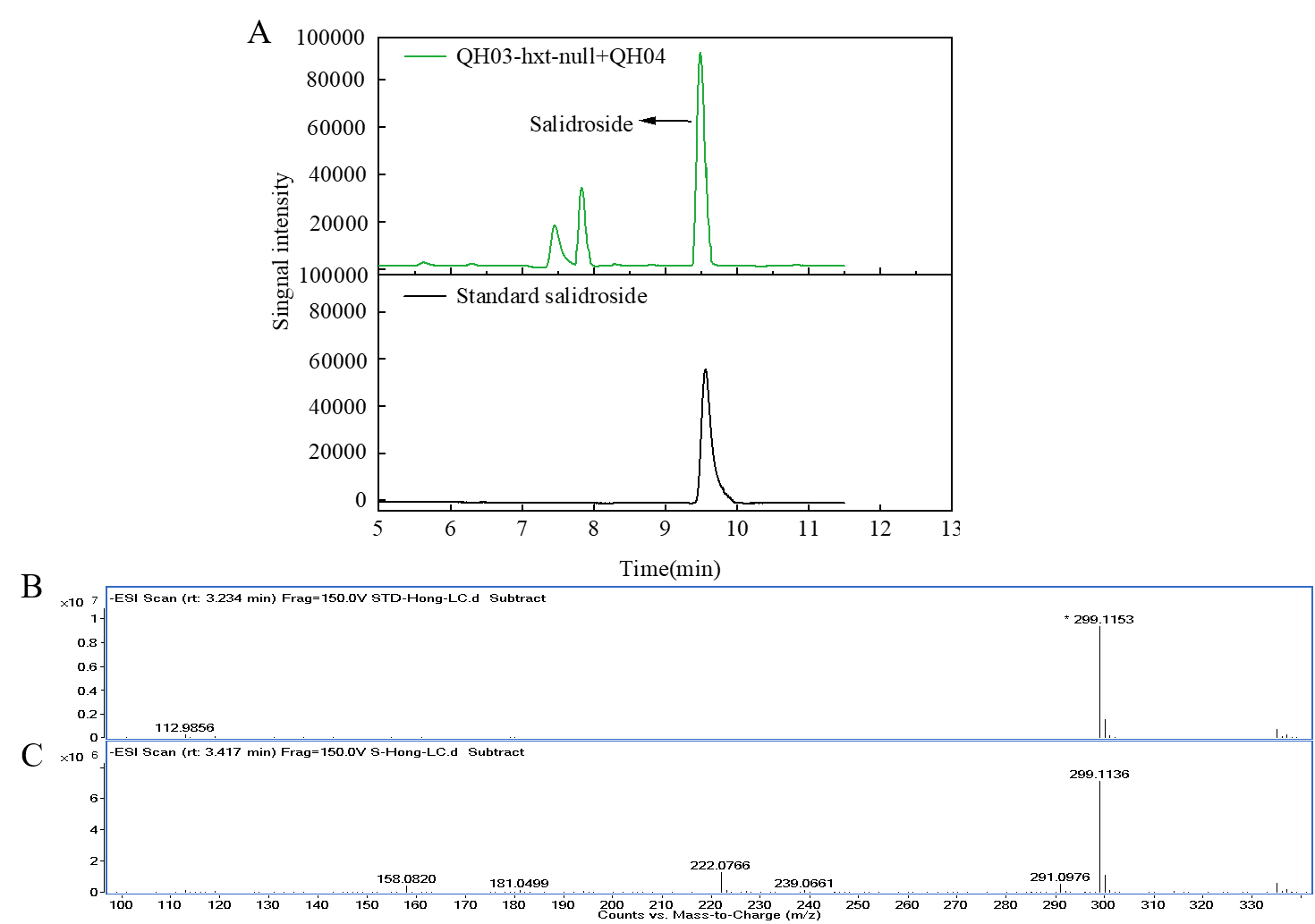


Figure. S3 A. HPLC analysis of fermentation broth of QH03 and QH04 strains coculture. B. The LC–MS/MS of the standard salidroside. C. The LC–MS/MS of the fermentation supernatant sample of QH03 and QH04 strains coculture. Strain coculture produced a major product that has identical retention time and yields identical ([M]-) ions to standard salidroside and the primary ion fragment at m/z 299([M]-) corresponds to salidroside with molecular weight of 300.