

Supporting Information 1

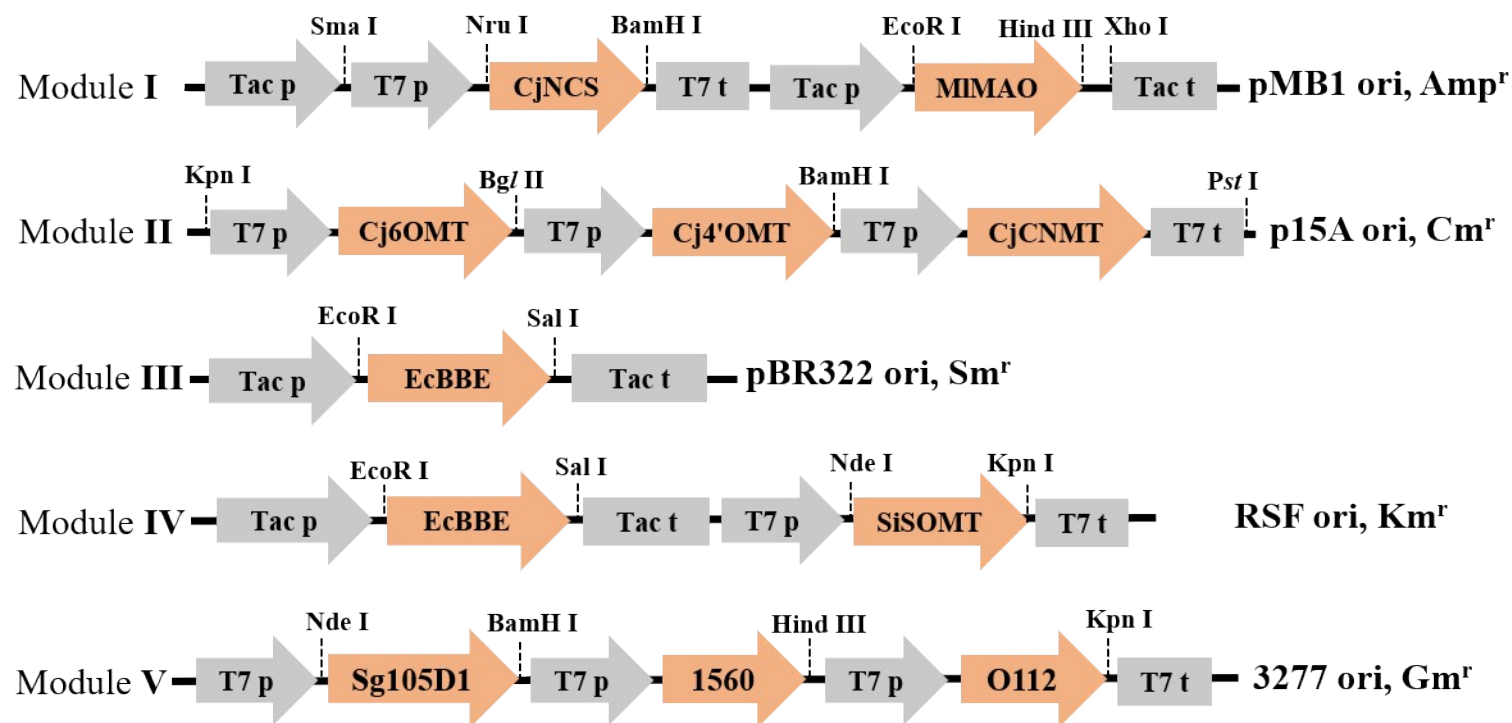


Fig. S 1. Schematic diagram of strain constructs.

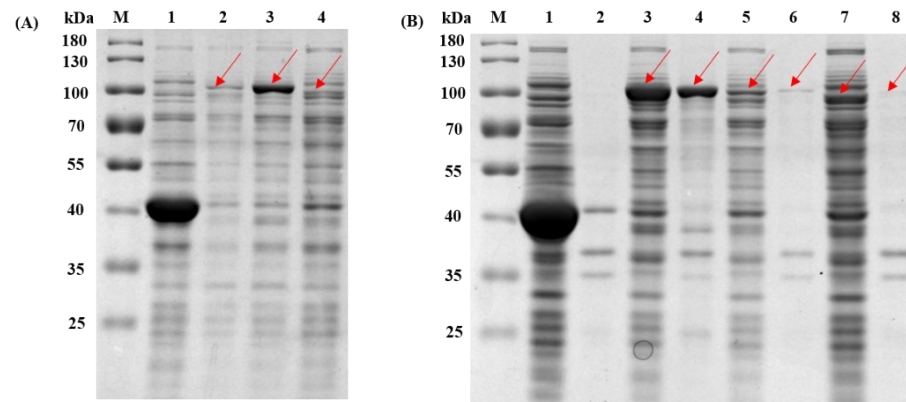


Fig. S 2. BBE protein expression from different sources in *E. coli*. (A) Whole-cell, M: marker; Lane 1: pMAL-c4x (vector control); Lane 2: SiBBE; Lane 3: EcBBE, and Lane 4: PsBBE. Soluble and insoluble. (B) M: marker; Lane 1: the supernatant of pMAL-c4x (vector control); Lane 2: the pellet of pMAL-4x (vector control); Lane 3: the supernatant of EcBBE; Lane 4: the pellet of EcBBE; Lane 5: the supernatant of SiBBE; Lane 6: the pellet of SiBBE; Lane 7: the supernatant of PsBBE, and Lane 8: the pellet of PsBBE.

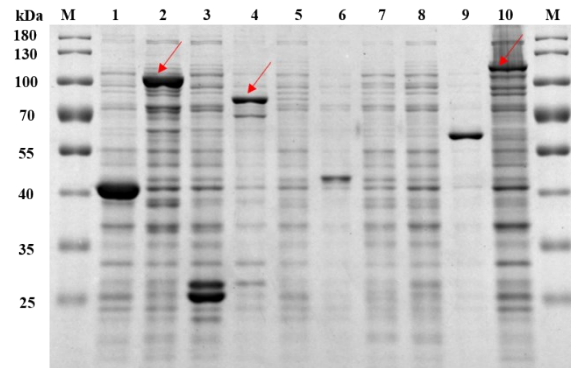


Fig. S 3. BBE protein expression with different tags in *E. coli* (whole cell). M: marker; Lane 1: pMAL-c4x (control; MBP \approx 40 kDa); Lane 2: M-EcBBE; Lane 3: pGEX6p-1 (control; GST \approx 26 kDa); Lane 4: 6P-EcBBE; Lane 5: pACYCDuet-1(control); Lane 6: CYC-EcBBE; Lane 7: pQE30 (control); Lane 8: 30-EcBBE; Lane 9: pCold TF (control, Trigger Factor \approx 58 kDa) and Lane 10: TF-EcBBE.

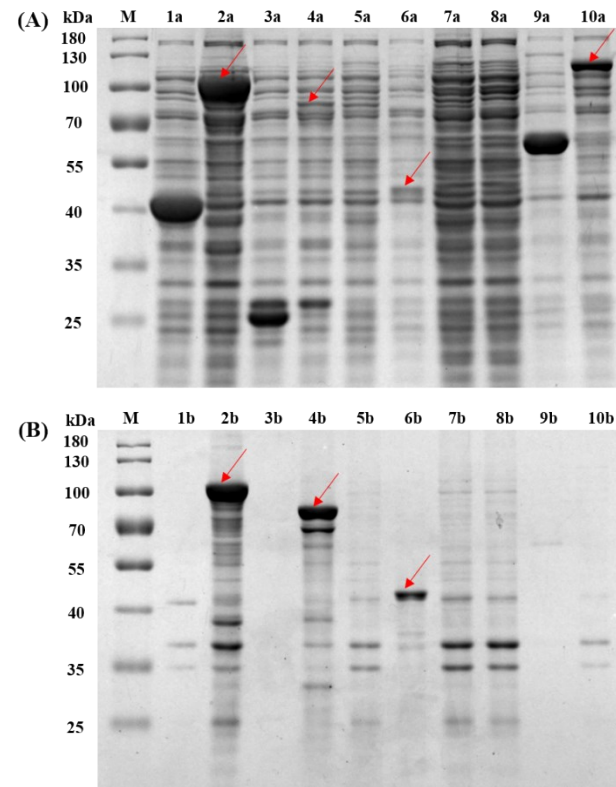


Fig. S 4. BBE protein expression with different tags in *E. coli* (soluble and insoluble). M: marker; Lane 1a: the supernatant of pMAL-c4x; Lane 1b: the pellet of pMAL-c4x; Lane 2a: the supernatant of M-EcBBE; Lane 2b: the pellet of M-EcBBE; Lane 3a: the supernatant of pGEX6p-1; Lane 3a: the pellet of pGEX6p-1; Lane 4a: the supernatant of 6P-EcBBE; Lane 4b: the pellet of 6P-EcBBE; Lane 5a: the supernatant of pACYCDuet-1; Lane 5b: the pellet of pACYCDuet-1; Lane 6a: the supernatant of CYC-EcBBE; Lane 6b: the pellet of CYC-EcBBE; Lane 7a: the supernatant of pQE30; Lane 7b: pellet of pQE30; Lane 8a: the supernatant of 30-EcBBE; Lane 8b:

the pellet of 30-EcBBE; Lane 9a: the supernatant of pCold TF; Lane 9b: the pellet of pCold TF; Lane 10a: the supernatant of TF-EcBBE and Lane 10b: the pellet of TF-EcBBE.

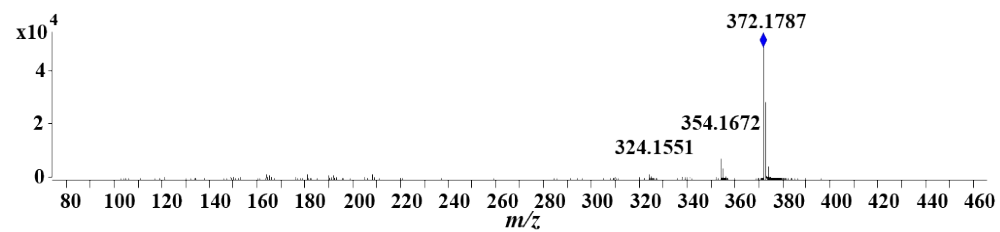


Fig. S 5. HPLC-Q-TOF-MS/MS identification of the 15.7 min peak from the strain BL21-105D1-1-1.

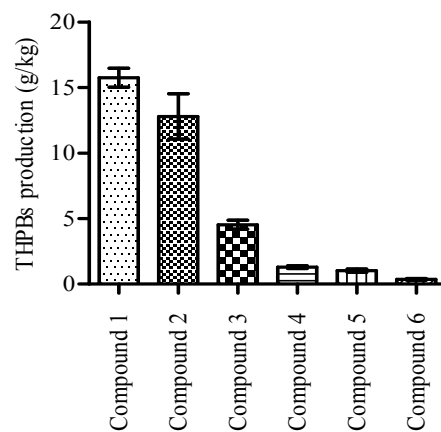


Fig. S 6. The dry cell weight yield of THPBs from *E. coli*.

Table S1. Analysis of BBE protein expression and activity.

| Various sources / various plasmids | Total protein (volume) | Soluble (%) | Insoluble (%) | Conversion rate (%) |
|------------------------------------|------------------------|-------------|---------------|---------------------|
| M-EcBBE (before optimization) | 53255760 | 58.94 | 41.06 | 32.75 |
| M-SiBBE | 9451860 | 74.95 | 25.05 | 1.38 |
| M-PsBBE | 8718960 | 85.38 | 14.62 | 3.47 |
| M-EcBBE(after optimization) | 87142172 | 42.34 | 57.66 | 78.90 |
| 6P-EcBBE | 43219253 | 12.24 | 87.76 | 18.65 |
| CYC-EcBBE | 3929686 | 69.93 | 30.07 | 1.09 |
| 30-EcBBE | 1019925 | 28.89 | 71.11 | 1.52 |
| TF-EcBBE | 25361490 | 82.00 | 18.00 | 1.93 |

Table S 2. HPLC-DAD-Q-TOF MS data for the reaction products.

| Compounds | t _R (min) | Molecular Formula | [M+H] ⁺ | Error (ppm) | Fragment Ions | Identification |
|-----------|----------------------|---|--------------------|-------------|--|---------------------------|
| Substrate | 3.15 | C ₈ H ₁₁ NO ₂ | 154.0857 | 3.63 | 137.0594, 119.0488, 109.0643 | dopamine |
| product 1 | 9.17 | C ₁₆ H ₁₇ NO ₄ | 288.1240 | -3.36 | 164.0704, 143.0491, 123.0440, 115.0542 | (S)-norlaudanosoline |
| product 2 | 13.88 | C ₁₉ H ₂₃ NO ₄ | 330.1705 | -1.60 | 239.1068, 207.0807, 192.1019, 137.0593 | (S)-reticuline |
| product 3 | 15.23 | C ₁₉ H ₂₁ NO ₄ | 328.1555 | -3.56 | 296.1227, 178.0861, 163.0622, 151.0737 | (S)-scoulerine |
| product 4 | 17.91 | C ₂₀ H ₂₃ NO ₄ | 342.1691 | 2.59 | 178.0852, 165.0901, 107.0476 | (S)-tetrahydrocolumbamine |
| product 5 | 19.30 | C ₂₁ H ₂₅ NO ₄ | 356.1856 | 0.10 | 192.1019, 165.0910, 131.0727 | (S)-tetrahydropalmatine |
| product 6 | 17.05 | C ₂₀ H ₂₃ NO ₄ | 342.1686 | 4.06 | 192.1007, 165.0898 | (S)-corydalmine |

Table. S 3 Effects of substrate on the growth of *E. coli*.

| Compound 5 (mg/mL) | cfu ($\times 10^6$) |
|--------------------|-----------------------|
| 0 | 162 |
| 0.1 | 154 |
| 0.2 | 148 |
| 0.5 | 132 |
| 1 | 117 |
| 2 | 96 |
| 3 | 54 |
| 4 | 31 |

Supporting Information 2. Nucleotide sequences of enzymes involved in alkaloids biosynthesis.

SiSOMT 1050bp

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Cj6OMT, 1041bp

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Cj4'OMT, 1053bp

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CjCNMT, 1077bp

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EcBBE 1545bp, After codon optimization

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CjNCS 558bp, After codon optimization

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