**Optimized sampling protocol for mass spectrometry-based Metabolomics in *Streptomyces***

List of supplementary Tables in supplementary file 1

**Table S1.** Details of quenching solutions and extraction procedures. Q1-Q5 denotes 5 quenching experiments and E1-E3 denotes 3 extraction procedures.

**Table S2**. The gradient and flow rate for the determination of nucleotides.

**Table S3.** The gradient and flow rate for the determination of coenzymes.

**Table S4.** Reproducibility is assessed by %RSD in different extraction and quenching conditions.

**Table S5.** Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution and thawing-freezing (TF) in liquid nitrogen and cryostat at -30℃ within 50%(v/v) methanol for three cycles as extraction solutions. IsoaB: isoamylol with base solution 5:1(v/v) at -30℃ and Meth60: 60% methanol at -30℃, respectively. Base solution was made up of acetone: ethanol= 1:1 (mol/mol). Multiples were calculated by IsoaB/Meth60.

**Table S1**

Details of quenching solutions and extraction procedures. Q1-Q5 denotes 5 quenching experiments and E1-E3 denotes 3 extraction procedures.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Numbers | | Quenching solution A:B=5:1, v/v | | | | Extraction | | |
|  | A | | B | Abbreviation |  | | Abbreviation | | |
| Q1 | Acetone | | Acetone: ethanol=1:1, mol/mol | AceB | Thawing-Freezing | | | TF | |
| Q2 | Isoamylol | | Acetone: ethanol=1:1, mol/mol | IsoaB | Thawing-Freezing | | | TF | |
| Q3 | Propanol | | Acetone: ethanol=1:1, mol/mol | ProB | Thawing-Freezing | | | TF | |
| Q4 | Methanol | | Acetone: ethanol=1:1, mol/mol | MethB | Thawing-Freezing | | | TF | |
| Q5 | Methanol 60%(v/v) | | Methanol 60%(v/v) | Meth60 | Thawing-Freezing | | | TF | |
| E1 | Isoamylol | | Acetone: ethanol=1:1, mol/mol | IsoaB | Boiling ethanol | | | BE | |
| E2 | Isoamylol | | Acetone: ethanol=1:1, mol/mol | IsoaB | Thawing-Freezing | | | TF | |
| E3 | Isoamylol | | Acetone: ethanol=1:1, mol/mol | IsoaB | Grinding | | | G | |

**Table S2**

The gradient and flow rate for the determination of nucleotides.

|  |  |  |  |
| --- | --- | --- | --- |
| Time | A | B | Rate |
| [min] | [%] | [%] | [mL/min] |
| 0 | 90 | 10 | 0.1 |
| 5 | 77 | 23 | 0.1 |
| 8 | 77 | 23 | 0.1 |
| 8.1 | 90 | 10 | 0.2 |
| 19 | 90 | 10 | 0.2 |
| 19.1 | 90 | 10 | 0.1 |
| 20 | 90 | 10 | 0.1 |

**Table S3**

The gradient and flow rate for the determination of coenzymes.

|  |  |  |  |
| --- | --- | --- | --- |
| Time | A | B | Rate |
| [min] | [%] | [%] | [mL/min] |
| 0 | 90 | 10 | 0.2 |
| 5 | 60 | 40 | 0.2 |
| 8 | 60 | 40 | 0.2 |
| 10 | 90 | 10 | 0.2 |
| 15 | 90 | 10 | 0.2 |

**Table S4**

Reproducibility is assessed by %RSD in different extraction and quenching conditions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | IsoaB | ProB | MethB | AceB | Meth60 | BE | TF | G |
| AA | 2.17 | 2.57 | 4.80 | 7.97 | 3.28 | 1.32 | 1.64 | 1.74 |
| OA | 12.81 | 12.70 | 13.93 | 17.11 | 14.78 | 9.64 | 5.08 | 7.85 |
| PS | 15.49 | *16.75* | 15.29 | 16.62 | 17.62 | 15.46 | 10.34 | 14.96 |
| SA | 6.93 | 7.58 | 12.15 | 8.52 | 8.06 | 6.30 | 5.84 | 6.02 |
| Average | 9.35 | 9.90 | 11.54 | 12.55 | 10.94 | 8.18 | 5.72 | 7.64 |

**Table S5**

Intracellular metabolites in *Streptomyces* ZYJ-6. Based on IsoaB and Meth60 as the quenching solution and thawing-freezing (TF) in liquid nitrogen and cryostat at -30℃ within 50%(v/v) methanol for three cycles as extraction solutions. IsoaB: isoamylol with base solution 5:1(v/v) at -30℃ and Meth60: 60% methanol at -30℃, respectively. Base solution was made up of acetone: ethanol= 1:1 (mol/mol). Multiples were calculated by IsoaB/Meth60.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Number | Metabolites | IsoaB | Meth60 | Multiples |
| [-] | [-] | [μmol gDCW-1] | [μmol gDCW-1] | [-] |
| 1 | 6PG | 6.73 | 1.58 | 4.26 |
| 2 | AceCoA | 0.44 | 0.60 | 0.73 |
| 3 | ADP | 0.89 | 0.65 | 1.36 |
| 4 | aKG | 57.24 | 4.96 | 11.54 |
| 5 | AMP | 3.80 | 5.27 | 0.72 |
| 6 | Asn | 15.29 | 3.71 | 4.12 |
| 7 | Asp | 5.05 | 2.49 | 2.03 |
| 8 | ATP | 4.94 | 0.13 | 38.40 |
| 9 | Cit | 2.84 | 0.88 | 3.23 |
| 10 | CoA | 21.60 | 12.67 | 1.70 |
| 11 | Ery | 7.25 | 0.62 | 11.67 |
| 12 | F6P | 3.12 | 1.72 | 1.82 |
| 13 | FBP | 1.65 | 0.70 | 2.35 |
| 14 | Fum | 6.51 | 1.18 | 5.51 |
| 15 | G3P | 5.63 | 2.76 | 2.04 |
| 16 | G6P | 6.95 | 3.92 | 1.77 |
| 17 | Gln | 170.85 | 38.22 | 4.47 |
| 18 | Glu | 211.34 | 114.51 | 1.85 |
| 19 | Gly | 17.75 | 8.93 | 1.99 |
| 20 | Ile | 50.28 | 7.25 | 6.94 |
| 21 | IMP | 2.54 | 1.82 | 1.39 |
| 22 | Leu | 33.49 | 4.89 | 6.86 |
| 23 | M6P | 9.96 | 6.06 | 1.64 |
| 24 | Mal | 4.84 | 0.07 | 72.05 |
| 25 | Manl | 94.25 | 89.74 | 1.05 |
| 26 | Met | 0.36 | 0.16 | 2.21 |
| 27 | Oaa | 26.05 | 3.24 | 8.04 |
| 28 | Orn | 21.17 | 4.37 | 4.85 |
| 29 | OX | 89.01 | 2.22 | 40.02 |
| 30 | PEP | 0.13 | 0.08 | 1.67 |
| 31 | Phe | 5.03 | 1.22 | 4.11 |
| 32 | Pro | 24.06 | 2.71 | 8.89 |
| 33 | Pyr | 4.60 | 1.05 | 4.38 |
| 34 | R5P | 22.61 | 1.50 | 15.04 |
| 35 | RL5P | 8.55 | 1.08 | 7.94 |
| 36 | S7P | 5.77 | 2.94 | 1.96 |
| 37 | SucCoA | 0.04 | 0.06 | 0.63 |
| 38 | Ser | 14.49 | 3.67 | 3.95 |
| 39 | Ska | 0.38 | 0.17 | 2.19 |
| 40 | Suc | 8.11 | 1.39 | 5.82 |
| 41 | T6P | 0.84 | 0.36 | 2.30 |
| 42 | Thr | 65.69 | 8.97 | 7.32 |
| 43 | Val | 96.67 | 11.34 | 8.53 |
| 44 | Xyl | 0.27 | 0.04 | 6.26 |