Supplementary Material

Supplementary experimental procedures

Sample Preparation Process for Metabolomics

1. Sample preparation

Brucella broth, the bacterial suspensions were adjusted to OD600 = 1.0 and then used for metabolite extraction.

(1) All samples were lyophilised and weighed to 15 mg, to which a total of 1 mL of pre-cooled methanol-water (V:V=4:1, containing mixed internal standard, 4 μg/mL) was added and transferred in two batches to a glass vial.

(2) Add 200 μL of chloroform and blow out with a pipette gun;

(3) Ultrasonic breakage in an ice bath, 500 W, for 6 min (6 s on, 4 s off);

(4) Transfer all the liquid into a centrifuge tube, ultrasonic extraction in an ice-water bath for 20 min, and leave at -40°C overnight;

(5) Centrifuge for 10 min (12000 rpm, 4℃), take 800 μL into the LC-MS injection vial to wave dry;

(6) Dissolve with 300 μL methanol-water (V:V=1:4), vortex for 30 s, sonicate for 3 min in an ice-water bath, and let stand at -40℃ for 2 hours;

(7) Centrifuge for 10 min (12000 rpm , 4°C), aspirate 150 μL of the supernatant with a syringe, filter it using a 0.22 μm organic-phase pinhole filter, transfer it to an LC injection vial, and store it at -80°C until LC-MS analysis.

(8) Quality control (QC) samples were prepared by mixing equal volumes of extracts from all samples.

(9) Note: All extraction reagents were pre-cooled at -20°C before use.

2. Liquid chromatography-mass spectrometry analysis conditions

The analytical instrument for this experiment was a liquid-mass spectrometry system consisting of a Waters ACQUITY UPLC I-Class plus/Thermo QE plus ultra-high performance liquid tandem high-resolution mass spectrometer.