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# Mass Spectrometry analysis and Molecular MS/MS network

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1 Works for me

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KEYWORDS

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## MATERIALS TEXT

### Materials

Glass jars with screw caps of the capacity of 1000 mL  
Beakers 100 - 600 mL  
pH meter  
Notebook  
Absorbent paper  
Tips of 10 µL, 200 µL, 1000 µL  
Permanent marker for labeling  
Gloves  
Dry extract  
Syringe-filter 0.22 µm  
Vials

### Reagents

methanol (HPLC grade)  
Formic acid 0.1%  
Acetonitrile

### Solutions

Sterile deionized water

### Other

Micropipette of 10 µL, 200 µL, 1000 µL  
Analytical balance  
Freezer  
Ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS) Thermo Scientific QExactive® Hybrid Quadrupole-Orbitrap Mass Spectrometer.  
Column (Stationary phase: Thermo Scientific Accucore C18 2.6 µm (2.1 mm x 100 mm)  
Xcalibur software (version 3.0.63) Thermo Fisher Scientific  
GNPS platform (<https://gnps.ucsd.edu/>)

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## UHPLC

- 1 Resuspend the dry extract in 1 mL of methanol (HPLC grade) and dilute 100 µL in 900 µL of methanol (HPLC grade).
- 2 Filter the final solution using a syringe-filter into vials
- 3 Perform Ultra-high pressure liquid chromatography-mass spectrometry (UHPLC-MS) analyses in a Thermo Scientific QExactive® Hybrid Quadrupole-Orbitrap Mass Spectrometer. Stationary phase: Thermo Scientific Accucore C-18 2.6 µm (2.1 mm x 100 mm)

- 4 Set the following parameters in the spectrometer: positive mode, capillary voltage at +3.5 kV; capillary temperature at 250 °C; S-lens of 50 V and m/z range of 133.40-2000.00
- 5 Perform tandem mass spectrometry (MS/MS) using normalized collision energy (NCE) of 30 eV and 5 precursors per cycle
- 6 Set the following conditions in the column mobile phase: 0.1% formic acid (A) and acetonitrile (B). Eluent profile (A: B) 0-10 min, gradient from 95:5 up to 2:98; held for 5 min; 15-16.2 min gradient up to 95:5; held for 8.8 min. Flow rate: 0.2 mL min<sup>-1</sup>
- 7 Inject 3 µL of each sample, then conduct operation and spectra analyses using Xcalibur software (version 3.0.63) developed by Thermo Fisher Scientific

#### GNPS Analysis

- 8 Create a molecular network for *Streptomyces* sp. using the online workflow at GNPS (<https://gnps.ucsd.edu/>) for molecular networking analysis.
- 9 Filter the data by removing all MS/MS peaks within  $\pm 17$  Da of the precursor m/z
- 10 Window filter the MS/MS spectra by choosing only the top 6 peaks in the  $\pm 50$  Da window throughout the spectrum
- 11 Cluster the data with MS-Cluster with a parent mass tolerance of 0.02 Da and an MS/MS fragment ion tolerance of 0.02 Da to create consensus spectra
- 12 Create a network where edges were filtered to have a cosine score above 0.5 and more than 5 matched peaks
- 13 Keep further edges between two nodes in the network only if each of the nodes appeared in each other's respective top 10 most similar nodes
- 14 Search the spectra comparing with those in the network GNPS' spectral libraries
- 15 Filter the library spectra in the same manner as the input data.
- 16 Keep all matches of network and library spectra that have a score above 0.5 and at least 5 matched peaks

