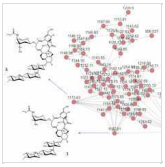

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Mass Spectrometry analysis and Molecular MS/MS network

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1 *Works for me* dx.doi.org/10.17504/protocols.io.br2fm8bn

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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 plataform (https://gnps.ucsd.edu/)

DISCLAIMER:

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)

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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^{-1}

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 ± 17 Da of the precursor m/z

10 Window filter the MS/MS spectra by choosing only the top 6 peaks in the ± 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 comparining 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

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