Jan 29, 2019

Leaf surface acylsugar extraction and LC-MS profiling - v1.0

eLife

DOI
dx.doi.org/10.17504/protocols.io.xj2fkqe

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DOI: dx.doi.org/10.17504/protocols.io.xj2fkqe

External link: https://doi.org/10.1126/sciadv.aaw3754

Protocol Citation: Yann-Ru Lou, Bryan Leong 2019. Leaf surface acylsugar extraction and LC-MS profiling - v1.0. **protocols.io https://dx.doi.org/10.17504/protocols.io.xj2fkqe**

Manuscript citation:
Leong BJ, Lybrand DB, Lou Y, Fan P, Schilmiller AL, Last RL, Evolution of metabolic novelty: A trichome-expressed invertase creates specialized metabolic diversity in wild tomato. Science Advances 5(4). doi: 10.1126/sciadv.aaw3754

Protocol status: Working
We use this protocol and it's working

Created: January 29, 2019

Last Modified: January 29, 2019

Protocol Integer ID: 19802

Keywords: leaf surface, acylsugar, sugar esters, extraction, LC-MS, Trichome, glandular, glycolipids

Abstract

- We use this protocol to extract and study acylsugars from glandular trichomes present on the surface of *Solanum lycopersicum* and *Solanum pennellii* plants as described in our manuscript "Evolution of metabolic novelty: a trichome-expressed invertase creates specialized metabolic diversity in wild tomato" (doi: https://doi.org/10.1101/502971).

- While this method is optimized for acylsugar extraction, it can be tweaked to use for other metabolites on the leaf surface.

- Below is a literature that is highly useful for acyslugar identification:
- Comparative structural profiling of trichome specialized metabolites in tomato (*Solanum lycopersicum*) and *S. habrochaites*: acylsugar profiles revealed by UHPLC/MS and NMR (doi: https://doi.org/10.1007/s11306-013-0585-y)

Guidelines

LC-MS Analysis

- If you are not familiar with LC-MS, we highly recommend getting on-site help from an experienced person. Do not attempt to set up LC-MS analysis merely based on this protocol.

Glassware

- Carefully wash the glassware used for LC-MS solvent and acylsugar extraction solution preparation to remove contaminants that might interfere with LC-MS analysis.
- Our standard protocol calls for six times thorough rinses with water to avoid detergents, and several round of rinsing with appropriate organic solvent. While other contamination might occur under different circumstances, we suggest checking with your LC-MS specialists or core facility manager for preferred cleaning protocol.

Extraction Tubes

- We use Micrew tube with O-ring seal (1.5 mL) extraction vial for our study. Snap-lid polyethylene microcentrifuge tubes are compatible too.

Solvent and Solution Storage

- We suggest always making fresh solvent and solution. However, in our experience, extraction buffer could be stored short term at 4℃ or -20℃ with no obvious issues. We did not carry out degradation curve and do not claim to know the best storage condition.

Acylsugar Identification

- For more information, please see:
- Evolution of metabolic novelty: a trichome-expressed invertase creates specialized metabolic diversity in wild tomato" (doi: https://doi.org/10.1101/502971).
- Comparative structural profiling of trichome specialized metabolites in tomato (*Solanum lycopersicum*) and *S. habrochaites*: acylsugar profiles revealed by UHPLC/MS and NMR (doi: https://doi.org/10.1007/s11306-013-0585-y)

Materials

MATERIALS

- Acetonitrile **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**34998
- Ascentis Express C18 2.7 µm HPLC column **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53823-U
- 2-propanol **J.T. Baker Catalog #**9084
- Telmisartan **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**T8949
- LC-MS clear vial (2 mL) **Restek Catalog #**21140
- LC-MS cap **J.G. Finneran Associates Inc Catalog #**5395F-09RB
- Formic acid (88%) **Fisher Scientific Catalog #**A119P-500
- Ammonium formate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**70221-25G-F
- DMSO **J.T. Baker Catalog #**9224
- Micrewtube with O-ring seal (1.5 mL) **Dot Scientific Catalog #**T332-4SPR
- Waters Acquity UPLC **Waters**
- Xevo G2-XS QToF MS **Waters**

For more information regarding the Waters UPLC and/or Xevo G2-XS QToF MS, please contact the MSU RTSF Mass Spectrometry core at: **RTSF.MassSpec@msu.edu**

Before start

- For each sample, label an extraction vial and a corresponding LC-MS glass vial
- When you are collecting samples, process an additional extraction vial and solvent as normally would to use as a blank for LC-MS
- Consider pre-weighing (before extraction) and re-weighing (after extraction) each labeled extraction vial to make measuring the leaf weight easier

Reagent preparation

1 Prepare the Acylsugar Extraction Solution (3:3:2 Acetonitrile:Isopropanol:water, 0.1% formic acid, 1 µM telmisartan (internal standard))

To prepare 1000 mL Acylsugar Extraction Solution, combine:

- 375 mL Acetonitrile
- 375 mL Isopropanol
- 250 mL ddH_2O
- 1136.38 µL Formic Acid (88%) (0.1% final conc.)
- 1000 µL 1 mM Telmisartan (dissolved in 100% DMSO; final conc. 1 µM)

Note

We suggest always making fresh solution. However, in our experience, extraction buffer could be stored short term at 4°C or -20°C with no obvious issues. We did not carry out degradation curve and do not claim to know the best storage condition.

Reagent preparation

2 Dispense 1 ml of Acylsugar Extraction Solution into the labeled extraction vial

Acylsugar extraction

3 Using a fine scissors or forceps, cut 1-2 small new leaflets and immediately place them in the designated extraction vial

Note

Clean the forceps and/or scissors with Acetonitrile and a Kimwipe between samples.

4 Tighten the cap well and gently agitate the tube on a rocker for 00:02:00

5 Remove the vial from the rocker and immediately pipette the extraction solution into the corresponding LC-MS glass vial and seal it with the cap.

Note

- Make sure the leaflet remains in the extraction vial.
- Acylsugars are not green, however, disturbance of chlorophyll and/or other metabolites might make the extraction solution turn green-ish looking. Based on our experience, this does not affect the extraction and qualification of acylsugars.

6
- Store the LC-MS vials at -20 °C.

For quantification purpose:
- Place uncapped extraction vials (containing leaf tissue) in a drying cabinet (~40°C) and discard the extraction vial cap.
- Weigh dried leaf tissue after 3 days or after thoroughly dried

LC-MS metabolite profiling

7 To analyze extraction on LC-MS, we use the following LC-MS system and methods:

- Waters Acquity UPLC
- Waters Xevo G2-XS QToF mass spectrometer

- Column: Ascentis Express C18 HPLC column (10 cm x 2.1 mm, 2.7 µm)
- Oven temp: 40 °C
- Flow rate: 0.3 mL/min

- Solvent A: 10 mM ammonium formate, pH 2.8
- Solvent B: 100% acetonitrile

Note

To prepare 1000 mL of 10 mM Ammonium Formate, pH 2.8:

- Add 630.6 mg of Ammonium Formate to 950 mL of water
- Adjust the pH of the solution to 2.8 using formic acid
- Add water up to 1000 mL

- Table 1. **7-min LC method**

	Time (min)	Solvent A %	Solvent B %
	0.00	95.0	5.0
	1.00	40.0	60.0
	5.00	0.0	100.0
	6.00	0.0	100.0
	6.01	95.0	5.0
	7.00	95.0	5.0

- Table 2. **21-min LC method**

	Time (min)	Solvent A %	Solvent B %
	0.00	95.0	5.0
	3.00	40.0	60.0
	15.00	0.0	100.0
	18.00	0.0	100.0
	18.01	95.0	5.0
	21.00	95.0	5.0

Note

- If you are not familiar with LC-MS, we highly recommend getting on-site help from an experienced person.

8 To analyze extraction on LC-MS, we use the following MS settings:

For **negative ion-mode** electrospray ionization:
Capillary voltage: 2.00 kV
Source temperature: 100°C
Desolvation temperature: 350°C
Desolvation nitrogen gas flow rate: 600 liters/h
Cone voltage: 35V
Mass range: m/z 50 to 1000 with spectra accumulated at 0.1 seconds/function

Collision energies:
Function 1: 0V
Function 2: 15V
Function 3: 35V

For **positive ion-mode** electrospray ionization:

Capillary voltage: 3.00 kV
Source temperature: 100°C
Desolvation temperature: 350°C
Desolvation nitrogen gas flow rate: 600 liters/h
Cone voltage: 35V
Mass range: m/z 50 to 1000 with spectra accumulated at 0.1 seconds/function

Collision energies:
Function 1: 0V
Function 2: 15V
Function 3: 45V

Fore more detail, please refer to the experiment files:

ESI- experiment file.txt
ESI+ experiment file.txt

Note

- If you are not familiar with LC-MS, we highly recommend getting on-site help from an experienced person.

9 For a list of manuscripts with useful information regarding acylsugar identification (masses, fragmentation patterns and quanitifcation methods), please refer to the description page of this protocol.