Analysis and characterization of the carbohydrate fraction of Aiptasia and coral tissue using targeted GC-MS

Hagen Gegner,Michael Ochsenkuehn,Christian Voolstra

Abstract

The description and identification of metabolites using Gas Chromatography - Mass Spectrometry (GC-MS) is a powerful tool to study the cnidarian-dinoflagellate symbiosis. The applications range from first descriptions of metabolite profiles to the identification of specific metabolic responses (e.g. biomarkers) of the cnidarian metaorganism ('holobiont'), including its associated symbionts, under stress.

The following step-by-step protocol is optimized to characterize the carbohydrate fraction from Aiptasia anemones or coral fragments, as well as their algal symbionts, using a targeted GC-MS approach. The protocol was previously used in Ochsenkühn et al. (2017) where it identified the following carbohydrates (floridoside, inositol, mannitol, glucose, glycerol, galactose, ribose and fructose) as well as some amino acids (glycine, alanine, valine and proline).

In addition to the step-by-step protocol for sample preparation and derivatization, we provide detailed settings for the Agilent GC-MS system (GC (Agilent 7890A) and MS (Agilent 5975C)) in a separate document.

Citation: Hagen Gegner,Michael Ochsenkuehn,Christian Voolstra Analysis and characterization of the carbohydrate fraction of Aiptasia and coral tissue using targeted GC-MS. **protocols.io**
dx.doi.org/10.17504/protocols.io.s7fehjn
Published: 03 Sep 2018

Guidelines

It is crucial that all steps, until the derivatization, are performed on ice to minimize degradation of metabolites. Normalization is essential for GC-MS data, as such, two options (dry-weight and total protein content) are included in this protocol.

Materials

double distilled water (ddH2O) by Contributed by users

Ethanol, absolute 99.8% 10342652 by Contributed by users

Methoxamine (MOX) Reagent TS-45950 by Thermo Fisher Scientific

N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) 69479 by Sigma Aldrich

4-Hydroxybenzoic acid 240141 Aldrich by Sigma Aldrich

Protocol

Disclaimer

Step 1.

This protocol is a step-by-step guide to extract and analyze the carbohydrate fraction of Aiptasia and coral tissue. We included further notes to separate the algal symbiont fraction from the host fraction.

Normalization is essential for GC-MS data, as such two options (dry-weight and total protein content) are included in this protocol. Depending on the method chosen follow the respective 'STEP-CASE' normalization to dry-weight and/or to protein content.

To minimize degradation of metabolites, it is advised to work quickly and reduce waiting times. Further, it is crucial that that all steps, until the derivatization, are performed on ice/in the cold.

If you are interested in other protocols related to the model organism Aiptasia:

PROTOCOL

. Getting started with the Aiptasia-Symbiodinium Model System

CONTACT: Aiptasia Model

Materials

Step 2.

Chemicals

- MOX reagent 2% methoxamine HCL in pyridine (e.g. Thermo scientific)
- MSTFA, 1% TMCS (10x1 ml. (e.g. Sigma 69479-10X1ML))
- 4-Hydroxybenzoic acid (ISTD = Internal standard)
- Absolute ethanol ≥99.8% (GC)

- Standards for quantification ≥99.8% (GC) (This depends on the compounds targeted)
    - We use: Glucose, Mannose, Succrose, Glycine

Consumables

- Falcon tubes (50ml)
- GC sample vials with Teflon cap (2ml)
- Glass syringe e.g. Hamilton® syringe, 700 series
- Syringe filters - 0.2 micron (13 mm diameter)
- Beckman bottles for Beckmann Coulter centrifuge
- GC vial glass inlets (0.2ml)
- Plastic syringes (1ml)
- Parafilm
- Kim wipes (or any other tissue)

Equipment

- GC/MS system
    - GC (Agilent 7890A)
    - MS (Agilent 5975C)
    - Autosampler (Agilent 7693)
- Concentrator system (Labconco Centrivap Complete)
- Ultracentrifuge (Beckmann Coulter Avanti J-26 XP)
- FreezeDryer (Ultradry)
- Ultrasonicator (Branson digital sonifier)
- Homogenizer (MicroDisTec MDT 125) (if needed)
- Centrifuge (Eppendorf 5415 R)
- Thermoblock fitting for GC vials
- Fume hood

Sample collection

Step 3.

At the end of your experiment:

- Rinse anemones or coral fragments with ddH2O to reduce the salt load
- Transfer anemones to cryotubes or in the case of coral fragments to a falcon tube or wrap in aluminum foil and snap freeze in liquid nitrogen

Sample collection

Step 4.

Store at -80 °C or continue with the protocol

Decide on the normalization method by following the step-case below:

- to protein content, directly follow the rest of the protocol
- to dry-weight, follow the steps and then go back to step-case 'normalization to protein content' to continue with the rest of the protocol

Sample preparation (dry-weight)

Step 5 - Normalization to dry-weight.

Thaw samples on ice and add ddH2O

- For Aiptasia add 1ml ddH2O
- For corals use an airbrush to remove the tissue or crush/pulverize fragments in liquide nitrogen and add up to 15ml ddH2O (the more you use the longer it takes in the next steps)

Sample preparation (dry-weight)

Step 6 - Normalization to dry-weight.

Transfer samples to a new, pre-weighed, falcon tube

Sample preparation (dry-weight)

Step 7 - Normalization to dry-weight.

Lyophilize samples over night using an type of lyophilizer (e.g. Ultradry)

Sample preparation (dry-weight)

Step 8 - Normalization to dry-weight.

After lyophilizing weigh the falcon tube + dried sample for later normalization to dry-weight

Sample preparation (dry-weight)

Step 9 - Normalization to dry-weight.

Continue with STEP-CASE: Normalization to total protein content and follow the rest of the protocol.

Sample preparation

Step 5 - Normalization to protein content.

Thaw samples on ice and add ddH2O

- For Aiptasia add 1ml ddH2O
- For corals use an airbrush to remove the tissue or crush/pulverize fragments in liquide nitrogen (if not done already)
    - add up to 15ml ddH2O (the more you use the longer it takes in the next steps)

Sample preparation

Step 6 - Normalization to protein content.

Sonicate using a tip ultrasonicator (e.g. Branson digital sonifier) on ice at 7 watts for 2min until

everything is dissolved.

Sample preparation

Step 7 - Normalization to protein content.

Remove cell debris by centrifuging at 4000g for 20min at 4 °C

Keep all samples on ice.

Sample preparation

Step 8 - Normalization to protein content.

Move supernatant to Beckman tube (50ml) and add 9 parts of absolute ethanol (cold) to 1 part of sample

Sample preparation

Step 9 - Normalization to protein content.

Leave them for 20min in a 4 °C fridge or on ice tp precipitate DNA/RNA/Protein

SAFETY INFORMATION

Balance the tubes for the ultracentrifuge, (+-0.05mg is ok)

Sample preparation

Step 10 - Normalization to protein content.

Centrifuge precipitate at 20.000g for 20min at 4 °C in Beckman centrifuge

PROTOCOL

. Protein extraction from Aiptasia

CONTACT: Jason Presnell

Step 10.1.

Prepare anemones by placing in the incubator in artificial seawater (ASW) 3-4 days prior to extraction. Be sure to change out the (ASW) each day to remove any residual debris.

Step 10.2.

Transfer 4-5 large anemones to a small tissue grinder on ice with 0.5-1 mL of chilled extraction buffer. Upon removal from their original container, anemones should be blotted on Kimwipes/weigh paper to remove as much water as possible.

Step 10.3.

Transfer homogenate to a 1.5 mL tube and centrifuge at 14,000xg for 15 minutes at 4°C. This

step will pellet the dinoflagellates and cell debris, while the protein will be in the supernatant.

Step 10.4.

Remove the supernatant (be careful not to disturb the white lipid layer!) and place in a new tube.

Step 10.5.

Determine the concentration of your protein (we used the Bradford assay).

Step 10.6.

Aliquot your protein into 100 µl volumes and store in the -80°C freezer until further use. Freeze-thaw cycles should be avoided and protein can be kept for a short period in the fridge on ice.

Sample preparation

Step 11 - Normalization to protein content.

Transfer the supernatant to a new falcon tube

Sample preparation

Step 12 - Normalization to protein content.

Put a kim wipe on the tubes lid, make holes in the lid and cool them down to -80C

(optinal: use Kim wipes and parafilm)

After preparation start the lyophilization (e.g. Ultradry lyophilizer)

DURATION
24:00:00 : minimum lyophilization
SAFETY INFORMATION
Cool samples down (-80 C) - otherwise the falcon tubes will overflow in the lyophilizer

Sample preparation

Step 13 - Normalization to protein content.

Possibility to pause the protocol and store dried samples

Derivatization (preparation)

Step 14 - Normalization to protein content.

Preparation of:

1. GC vials with smaller labels
2. Heated Thermo Block for GC vials
3. Concentrator system (max. 40 °C) Coldtrap (on) time 99=continuous (Labconco Centrivap

Complete)
4. Internal standard hydroxyl benzoic acid (ISTD) (1 ug/ul HBA in ddH2O)
5. Standards for quantification (that depends on the targeted compounds for quantification)

Derivatization (preparation)

Step 15 - Normalization to protein content.

Suspend dried samples in 500µl of ddH2O (its up to you, vials can hold 2ml)

- vortex vigorously

Derivatization (preparation)

Step 16 - Normalization to protein content.

Transfer suspended sample to a pre-labelled GC Vial

Derivatization (preparation)

Step 17 - Normalization to protein content.

Add 10µl of internal ISTD

- Add the internal Standard to all samples (including your calibration curves of standards for quantification)

Derivatization (preparation)

Step 18 - Normalization to protein content.

Dry samples using a Concentrator System (e.g. Labconco Centrivap Complete)

Derivatization (preparation)

Step 19 - Normalization to protein content.

Store samples

- Keep it dry, make sure lid is on and store in a vacuum

Derivatization (reaction)

Step 20 - Normalization to protein content.

Preparation of:

1. Thermoblock for GC vials
2. MOX (2% methoxamine HCL in pyridine)
3. MSTFA (MSTFA, 1% TMCS) (you can also use BSTFA))
4. Hamilton syringes (100µl)
5. Plastic syringes
6. Syringe Filters
7. Glass inserts for GC vials
8. Teflon lids for vials

SAFETY INFORMATION

MOX and MSTFA are volatile and toxic (corrosive) wear goggles, two layers of gloves and work under a fume hood. If a spill occurrs immediately switch gloves.

Derivatization (reaction)

Step 21 - Normalization to protein content.

Add 50µl of MOX using a Hamilton syringe to the dried samples and close vials tightly

Derivatization (reaction)

Step 22 - Normalization to protein content.

put samples in the thermo block for 1h at 75 °C

Derivatization (reaction)

Step 23 - Normalization to protein content.

Remove samples from the thermo block and add 100µl of MSTFA using a Hamilton syringe

- Have a second syringe to break open clumps so everything is accessible for the MSTFA
- vortex

Derivatization (reaction)

Step 24 - Normalization to protein content.

Close lids tightly and leave again for 1h in the 75 °C thermo block

Derivatization (reaction)

Step 25 - Normalization to protein content.

After 1 h, particles can be removed by syringe filtration through 0.2-micron (13 mm diameter) filters and transfer 120µL to glass inserts placed inside GC vials

Derivatization (reaction)

Step 26 - Normalization to protein content.

The vials are closed with PTFE septa screw caps or crimp caps and stored at -80 °C until measurement

GC-MS measurement

Step 27 - Normalization to protein content.

- On the day of the measurement remove samples from the freezer and let them reach room temperature before measuring.
- Measure in batches of ca. 10 samples and start with quality control and calibration curves first.
- **Find the settings for the GC-MS attached in an extra document.**

Good luck.

Sample preparation (Protein content normalization)

Step 5.

Warnings

The derivatization reaction steps have to be done in a fume hood as both reagants (MOX and MSTFA) are volatile and toxic (corrosive).

Wear goggles and a double layer of gloves and remove directly in case of any spill.