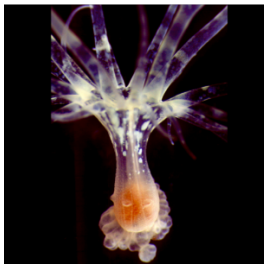
Sep 03, 2018

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



DOI
dx.doi.org/10.17504/protocols.io.s7fehjn

Hagen Gegner[1], Michael Ochsenkuehn[2], Christian R Voolstra[1]

[1]King Abdullah University of Science and Technology; [2]NYUAD

reefgenomics | Aiptasia Symbiodiniacea... | 1 more workspace

Hagen Gegner

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.s7fehjn

External link: http://advances.sciencemag.org/content/3/8/e1602047

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

Manuscript citation:
Ochsenkühn, M. A., Röthig, T., D'Angelo, C., Wiedenmann, J. and Voolstra, C. R. (2017). The role of floridoside in osmoadaptation of coral-associated algal endosymbionts to high-salinity conditions. *Sci. Adv.* **3**, e1602047.

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

Created: September 02, 2018

Last Modified: September 03, 2018

Protocol Integer ID: 15303

Keywords: GC-MS, Symbiosis, Salinity, Carbohydrates, Metabolomics, coral, aiptasia, sugars, mass-spectrometry

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.

Attachments

DOCX

GC-MS settings.docx

81KB

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

MATERIALS

- double distilled water (ddH2O)
- Ethanol, absolute 99.8% **Catalog #**10342652
- Methoxamine (MOX) Reagent **Thermo Fisher Scientific Catalog #**TS-45950
- N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**69479
- 4-Hydroxybenzoic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**240141 Aldrich

Safety 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.

Disclaimer

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

NAME

Getting started with the Aiptasia-Symbiodinium Model System

CREATED BY

Aiptasia Model

PREVIEW

Materials

2

Chemicals

- MOX reagent 2% methoxamine HCL in pyridine (e.g. Thermo scientific)
- MSTFA, 1% TMCS (10×1 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

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

Note

Salts may interfere with the derivatization or GC measurement. Therefore, proper rinsing is advised.

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

STEP CASE

Normalization to dry-weight 5 steps

Sample preparation (dry-weight)

5 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)

Note

At this stage you can isolate the algal symbiont from the tissue by repeated centrifugation and washing steps.

See this publication for a protocol:

Ochsenkühn, M. A., Röthig, T., D'Angelo, C., Wiedenmann, J. and Voolstra, C. R. (2017). The role of floridoside in osmoadaptation of coral-associated algal endosymbionts to high-salinity conditions. *Sci. Adv.* **3**, e1602047.

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

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

Note

Cover the opening with a fitting pre-cut Kim Wipe piece and tighten with parafilm along the rim so the liquide can escape.

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

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