Mar 29, 2022

Characterization of osmoregulated periplasmic glucans with high resolution LCMS

lcmsmethods [1], Benjamin C. Orsburn[1], Colten Eberhard[1], Allison Daitsch[1], Namandje N. Bumpus[1]

[1]The Johns Hopkins University Medical School

1

dx.doi.org/10.17504/protocols.io.36wgq7djkvk5/v1

LCMSMethods.org Version 2

lcmsmethods

Characterization of osmoregulated periplasmic glucans with high resolution LCMS

DOI

dx.doi.org/10.17504/protocols.io.36wgq7djkvk5/v1

lcmsmethods , Benjamin C. Orsburn, Colten Eberhard, Allison Daitsch, Namandje N. Bumpus 2022. Characterization of osmoregulated periplasmic glucans with high resolution LCMS. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgq7djkvk5/v1

protocol ,

Mar 29, 2022

Mar 29, 2022

60042

1 **CharacterizationofOPGsQExactiveTextMethod.docx**

Positive_Top3_12_1_2021_high_mass.meth

Positive_Top3_2_14_2021.meth

These are optimized methods for the characterization of osmoregulated periplasmic glucans

as described in Allison Daitsch et al., 2022.
Briefly: All analysis was performed on a Dionex UHPLC and Q Exactive quadrupole Orbitrap system (Thermo Fisher). Two micrograms of each reaction and unreacted input was injected directly onto a HyperSil Gold C-18 2.1mm x 150mm reversed phase chromatography column. Analytes were separated using an increasing gradient that consisted of 0.1% formic acid in LCMS grade water as buffer B and 0.1% formic acid in LCMS grade acetonitrile as buffer B. Due to the hydrophilic nature of glucans, the gradient began with a 2-minute acquisition at 100% buffer A with a rapid ramp to 100% buffer B by 15 minutes before returning to baseline conditions for the remainder of the 20 minute experiment. The Q Exactive was operated in positive ionization mode using a data dependent acquisition method. An MS1 scan was acquired at 140,000 resolution with a scan range of 150 to 1500 m/z. The three most abundant ions from each MS1 scan were isolated for fragmentation using a three-step collision energy of 10, 30 and 100 and the fragment scans were obtained using 15,000 resolution. Ions with unassigned charge states or more than 3 charges were excluded from fragmentation. To prevent repeat fragmentation any ion within 5 ppm mass deviation of the selected ion was excluded from additional fragmentation for 30 seconds. All Thermo .RAW instrument files have been uploaded to the MASSIVE public repository under accession MSV000089142 The vendor .RAW files and processed results can be accessed during the review process using the following link: ftp://MSV000089142@massive.ucsd.eduand reviewer password EstG725

A second method with the suffix "high_mass" is a recommended method for the characterization of OPGs of higher m/z charge ratio. Both methods can be utilized in both positive ionization mode as provided here, or in negative ionization mode by changing the polarity of the Q Exactive acquisition method. Low mass OPGs of interest for this study were identified solely with the positive *top32142021* method. Higher mass and negative polarity ionization may be necessary for the characterization of alternate OPG specis.

Citation: lcmsmethods , Benjamin C. Orsburn, Colten Eberhard, Allison Daitsch, Namandje N. Bumpus Characterization of osmoregulated periplasmic glucans with high resolution LCMS https://dx.doi.org/10.17504/protocols.io.36wgq7djkvk5/v1