

Whole-cell data independent mass spectrometry proteomic approach to examining *Emiliana Huxleyi* exposed to 4 treatments of HHQ

Website: <https://www.bco-dmo.org/dataset/827912>

Version: 1

Version Date: 2020-10-29

Project

» [Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics](#) (HHQSignals)

Contributors	Affiliation	Role
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Abstract

Interactions between phytoplankton and bacteria play a central role in mediating oceanic biogeochemical cycling and microbial trophic structure in the ocean. The intricate relationships between these two domains of life are mediated via excreted molecules that facilitate communication and determine competitive outcomes. Yet, despite their predicted importance, identifying these secreted compounds and understanding their ecological significance has remained a challenge. A whole-cell data independent mass spectrometry proteomic approach was used to identify phytoplankton proteins crucial in competitive interactions with bacterial infochemicals.

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Dataset Description

Data Independent Acquisition Proteomics: *Emiliana huxleyi* exposed to 4 treatments of HHQ (2-heptyl-4-quinolone). All files are in the Proteome Exchange Data Archive and publically accessible at: <https://www.ebi.ac.uk/pride/archive/projects/PXD011560>

Methods & Sampling

Emiliana huxleyi cells were grown to 72 hours in each treatment and 4 biological replicates from individual culture bottles were isolated for proteomics. Cells were pelleted using centrifugation and lysed with a sonicating probe. Proteins were reduced, alkylated, and digested with trypsin after being soubilized in 6M urea (see Nunn et al. 2016). All samples were desalted using NEST group desalting columns. Mass spectrometry was completed on Thermo Lumos with NanoAquity inline. Column was 75 micron ID, packed to 30cm with Dr. Maisch C18 packing material.

All DDA analyses were completed in single runs on the Lumos. 130 minute total run time, gradient 2-35% ACN in 90 minutes. OT resolution 120,000, scan range 375-1575, Rf lens 35%, AGC Target 7e5 MS2: charge state 2-5, Top 20 MS2, AGC target 5e3, dynamic exclusion 30 s

DIA analyses were completed on each sample, and a pooled sample that included equal parts from each treatment and biological replicate were included. Library generation was completed with 6 GPFs covering the range of 400-1000 in 6 windows with 4 m/z isolation windows. Each sample was also analyzed with 90 minute gradient scan range of 400-1000 and covered isolation window of 8m/z.

Analyses performed by Brook L. Nunn and Miranda M Mudge at the University of Washington Proteomics Resource Center (<https://proteomicsresource.washington.edu/>).

Data Processing Description

Data Processing: The database for *Emiliania huxleyi* was downloaded 2018-10-10 from Uniprot and concatenated with contaminants. Peptide spectral libraries were generated with XCORDIA and each sample set with wide window analysis was searched against the spectrallibrary using EncyclopeDIA.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Thermo Scientific Orbitrap Fusion Lumos Tribrid mass spectrometer
Generic Instrument Name	Mass Spectrometer
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Collaborative Research: Building a framework for the role of bacterial-derived chemical signals in mediating phytoplankton population dynamics (HHQSignals)

Coverage: Bergen, Norway

NSF Award Abstract:

Bacteria and phytoplankton play a central role in the modification and flow of materials and nutrients through the marine environment. While it has been established that interactions between these two domains are complex, the mechanisms that underpin these interactions remain largely unknown. There is increasing recognition, however, that dissolved chemical cues govern these microbial interactions. This project focuses on establishing a mechanistic framework for how bacterially derived signaling molecules influence interactions between phytoplankton and bacteria. The quorum-sensing (QS) molecule, 2-heptyl-4-quinolone (HHQ) will be used as a model compound for these investigations. Previously published work suggests that exposure to very low levels of HHQ results in phytoplankton mortality. Gaining a mechanistic understanding of these

ecologically important interactions will help to inform mathematical models for the accurate prediction of the cycling of material through the marine microbial loop. This work initiates a new, hybrid workshop-internship undergraduate research program in chemical ecology, with a focus

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Interactions between phytoplankton and bacteria play a central role in mediating biogeochemical cycling and microbial trophic structure in the ocean. The intricate relationships between these two domains of life are mediated via excreted molecules that facilitate communication and determine competitive outcomes. Despite their predicted importance, identifying these released compounds has remained a challenge. The PIs recently identified a bacterial QS molecule, HHQ, produced by globally distributed marine gamma-proteobacteria, which induces phytoplankton mortality. The PIs therefore hypothesize that bacteria QS signals are critical drivers of phytoplankton population dynamics and, ultimately, biogeochemical fluxes. This project investigates the timing and magnitude of HHQ production, and the physiological and transcriptomic responses of susceptible phytoplankton species to HHQ exposure, and quantifies the influence of HHQ on natural algal and bacterial assemblages. The work connects laboratory and field-based experiments to understand the governance of chemical signaling on marine microbial interactions, and has the potential to yield broadly applicable insights into how microbial interactions influence biogeochemical fluxes in the marine environment.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657818

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