Amphi-enterobactins and related siderophore concentrations found in Vibrio harveyi supernatants and pellets from laboratory experiments in 2017

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Project
» Iron uptake by marine bacteria: regulation and function of weak and strong siderophores (Bacteria Iron Siderophores)

Contributors	Affiliation	Role
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Abstract
Amphi-enterobactins and related siderophore concentrations found in Vibrio harveyi supernatants and pellets from laboratory experiments in 2017. These data were published in McRose et al. (2018, Fig. 4).

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Coverage

Temporal Extent: 2017

Methods & Sampling

Sampling and analytical procedures:

V. harveyi cells were cultured at 30C with shaking at 200 RPM. Growth experiments were conducted using a fully chemically defined artificial seawater medium consisting of basic salts (3x10-1 M NaCl, 1.05x10-2 M CaCl2Ÿ2H2O, 5x10-2 M MgSO4Ÿ7H2O, 4.85x10-4 M H3BO3) as well as 1x10-4 M K2HPO4, 6.51x10-2 M glycerol, 2.65 x10-8 M riboflavin, 2.96 x 10-6 M thiamine and Aquil trace metals without added Fe. Aquil trace metals contain 100 M EDTA, background Fe concentrations were determined by inductively coupled plasma MS (ICP-MS) to be ~100 nM. Nitrogen was added as MEM essential and non-essential amino acids (Sigma M5550, 92 mL L-1 ; Sigma M7145, 46 mL L-1). All cells were pre-cultured for ~24 hours in low Fe medium before the start of experiments to exhaust background trace metal supplies.

For quantification of siderophores ~50 mL of V. harveyi culture was centrifuged at 16,000 xg for 6 minutes. Supernatant samples were decanted, filtered (0.2 m) and acidified with 0.1% formic acid. Samples were then extracted using Oasis HLB (Waters) columns with the following conditions: 20 mL methanol, 20 mL MilliQ H2O,

50 mL sample, 20 mL 0.03% trifluoroacetic acid, 10 mL 0.03% formic acid and final elution with 30 mL of 40% methanol. Cell pellets were extracted overnight (~18 hours) with 5 mL of 80% methanol with 0.1% formic acid. Four mL of the resulting supernatant was diluted to 20% methanol with acidic (0.1% formic acid) MilliQ and extracted using an HLB column: 20 mL methanol, 20 mL MilliQ, 16 mL sample, 20 mL MilliQ and elution with 30 mL of 100% methanol. Samples were dried under vacuum (SpeedVac, ThermoFisher) and resuspended in either 1 mL MilliQ (supernatants) or 1 mL of 80% methanol (pellets). Extracted samples were acidified (0.1% acetic acid and 0.1% formic acid) and analyzed using electrospray-ionization LC-MS (Agilent 6120, Agilent, Santa Clara, CA, USA), with a UV-vis diode array detector and a C18 column (Agilent 4 Eclipse Plus C18, 3.5 m, 4.6 mm x 100 mm). Injected samples (100 L) were separated using a gradient of solutions A and B (A: water, 1% formic acid, 1% acetic acid, 1% acetonitrile; solution B: acetonitrile, 1% formic acid, 1% acetic acid, 2% water; gradient 0-100% B) over 30 min, with a flow rate of 0.8 mL min-1 . Full-scan mass spectra were collected in both positive- and negative-ion (m/z=140-1400).

Location: Laboratory experiments conducted at Princeton University.

Data Processing Description

Peaks related to amphi-enterobactins were identified by a combination of their characteristic masses and their absorbance at 310 nm using MassHunter (Agilent).

BCO-DMO Data Manager Processing Notes:
* Imported data table from file "Vharveyi-SiderophoreConcentrations.csv" into the BCO-DMO data system.
* Renamed columns to meet BCO-DMO naming conventions: https://www.bco-dmo.org/page/bco-dmo-data-processing-conventions

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Data Files

File
vharveyi_conc.csv (Comma Separated Values (.csv), 1.92 KB) MD5:9e5255118a70dd884e1093eedfc6c8dd Primary data file for dataset ID 861154

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Related Publications

Lilley, B. N., & Bassler, B. L. (2000). Regulation of quorum sensing in Vibrio harveyi by LuxO and Sigma-54. Molecular Microbiology, 36(4), 940–954. doi:10.1046/j.1365-2958.2000.01913.x
Methods

McRose, D. L., Baars, O., Seyedsayamdost, M. R., & Morel, F. M. M. (2018). Quorum sensing and iron regulate a two-for-one siderophore gene cluster inVibrio harveyi. Proceedings of the National Academy of Sciences, 115(29), 7581–7586. doi:10.1073/pnas.1805791115
Results

Naka, H., Reitz, Z. L., Jelowicki, A. L., Butler, A., & Haygood, M. G. (2018). Amphi-enterobactin commonly produced among Vibrio campbellii and Vibrio harveyi strains can be taken up by a novel outer membrane protein FapA that also can transport canonical Fe(III)-enterobactin. JBIC Journal of Biological Inorganic Chemistry, 23(7), 1009–1022. doi:10.1007/s00775-018-1601-5
Methods

Zane, H. K., Naka, H., Rosconi, F., Sandy, M., Haygood, M. G., & Butler, A. (2014). Biosynthesis of Amphi-enterobactin Siderophores by Vibrio harveyi BAA-1116: Identification of a Bifunctional Nonribosomal Peptide Synthetase Condensation Domain. Journal of the American Chemical Society, 136(15), 5615–5618. doi:10.1021/ja5019942
Methods

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Related Datasets

IsReferencedBy

Morel, F. (2021) **High resolution mass spectra for amphi-enterobactin related siderophores from Vibrio harveyi from laboratory experiments in 2017.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-09-21 doi:10.26008/1912/bco-dmo.861194.1 [view at BCO-DMO]
Relationship Description: High-resolution mass spectra for amphi-enterobactin from this same experiment.

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Parameters

Parameter	Description	Units
Time	Sampling time from start of incubation (decimal hours).	hours
Strain	Vibrio harveyi strain used. BB120 is the wild type strain (BAA-1116), JAF548 has a mutation that prevents it from responding to quorum sensing molecules (see Lilley and Bassler, 2000)	unitless
Siderophore	Specific siderophore being quantified. Amph-Ent refers to m/z 965	unitless
Conc	Siderophore concentration	micromoles per liter (umol/L, uM)
STDEVSiderophore	Standard deviation of biological duplicates for Siderophore concentration ("Conc" column).	micromoles per liter (umol/L, uM)
OD500	Optical density of the culture measured at 500 nm	unitless
STDEVOD	Standard deviation of biological duplicates for optical density ("OD500" column).	unitless
FigRef	Citation and figure where data are published (see Related Publications for full citation)	unitless

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Instruments

Dataset-specific Instrument Name	Agilent 6120 LC-MS (Agilent, Santa Clara, CA, USA)
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

Iron uptake by marine bacteria: regulation and function of weak and strong siderophores (Bacteria Iron Siderophores)

Coverage: laboratory

NSF abstract:
Organic molecules that bind and transport iron are called siderophores. Because iron is an essential trace element for biological systems and exists at very, very low concentrations in the open ocean, siderophores perform a critical role in capturing iron for cellular function. It is known that marine bacteria can produce two different types of siderophores that either tightly bind iron or only weakly do so, with different ecological consequences. This researcher will leverage an exceptional career on metal-organism interactions to examine the unsolved question of exactly what environmental and biochemical conditions (for example the availability of iron) control bacterial production of various siderophores. Results will generate significant new understanding of a critical chemical oceanographic process, and cap this researcher's groundbreaking discoveries that have built to this project. Funding for this research will also support the advancement of women in science by both providing the highest quality training of a female scientist and providing the opportunity for her to host an oceanography booth at the Princeton Plasma Physics Lab's "Young Women in Science" conference.

This study will use Vibrio harveyi as a model organism to investigate a variety of questions surrounding the marine bacterial production of weak and strong siderophores. To start, the investigation will look into how siderophore production is controlled by varying iron availability and quorum sensing (i.e. a coordinated response correlated to population density and/or certain signaling molecules). This also includes in-depth investigation of the impact of life phase and biochemical changes with growth as they relate to coordinated use of weak and strong siderophores. Using established protocols for genetic manipulation of V. harveyi, the researcher plans to discover how varying combinations of weak and strong siderophores maximize the uptake of iron. The broader biogeochemical implications of this study to the field of chemical oceanography, with regard to the microbial use of, and cellular responses to, many essential micronutrients in the ocean would be to significantly influence understanding of elemental distributions beyond the specific study of iron and siderophore cycling in the ocean.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1657639

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