Siderophore concentrations found in supernatants of Azotobacter vinelandii str. OP, Azotobacter chroococcum str. B3, and Azotobacter chroococcum str. NCIMB 8003 from laboratory experiments in 2015

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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
Siderophore concentrations found in supernatants of Azotobacter vinelandii str. OP, Azotobacter chroococcum str. B3, and Azotobacter chroococcum str. NCIMB 8003 from laboratory experiments in 2015. These data were published in Zhang et al. (2016, Fig 6).

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Coverage

Temporal Extent: 2015

Acquisition Description

Sampling and analytical procedures:

Wild type Azotobacter chroococcum strain B3 (ATCC strain 7486) and strain NCIMB 8003 (ATCC strain 4412) were grown aerobically in a modified Burk's medium under diazotrophic conditions by shaking at room temperature as previously described.14,48 For HR-LC-MS analysis of siderophores, the availability of Fe was limited by addition of 100 M EDTA to 0.1 M FeCl3 for AC-B3, whereas a concentration of 5 M FeCl3 was added to achieve sufficient growth with AC-8003. To study the effect of Fe sources, Fe was added as (1) 100 M EDTA and 0.1 M FeCl3; (2) 100 M EDTA and 5 M FeCl3; and (3) precipitated amorphous Fe

oxides. Bacterial growth was monitored at an optical density (OD) of 620 nm (OD620nm).

To follow concentrations of siderophores in AC-B3, 1 mL sample aliquots were taken at different times throughout the growth, filtered through 0.2 m syringe filters, acidified (0.1% acetic acid and 0.1% formic acid) and analyzed by direct injection on a single quadrupole LC-MS system (Agilent 6120). The analysis of all siderophores, except for crochelin A, was performed with a C18 column (Agilent Eclipse Plus C18 3.5 m, 4.6mm x 100 mm) and a gradient of solution A (water + 0.1% FA + 0.1% acetic acid) and B (acetonitrile + 0.1% FA + 0.1% acetic acid; gradient from 0–100% A over 30 min; flow rate of 0.8 mL/min). To achieve sufficient retention on the same C18 column, the analysis of crochelin A required the use of the ion-pairing reagent heptafluorobutyric acid (HFBA) added to solutions A (water + 0.05% HFBA) and B (acetonitrile + 0.05% HFBA) with a gradient from 0–100% A over 30 min; flow rate of 0.8 mL/min. The column outflow was diverted to waste for the first 5.25 min ensuring that the sample was completely desalted before introduction into the mass spectrometer. Siderophores were quantified by single ion monitoring (SIM) using a list of target m/z values of the identified siderophores. For quantification, LC-MS peak areas were determined and converted to concentrations by calibration with standards of vibrioferrin A,16 amphibactins ACA, S, and crochelin A. For catechol siderophores and azotobactins, LC-MS and UV-vis peak areas were determined using MassHunter software (Agilent). Relative peak areas were converted to concentrations by calibration with isolated standards of vibrioferrin, 2,3-dihydroxybenzoic acid (DHBA), azotochelin, protochelin, and azotobactin d. Seven technical replicates of a spent medium siderophore mix ‘‘standard’’ showed relative standard deviations of <10% when present at concentrations above 0.5 M. The detection limit of vibrioferrin A, amphibactins, and crochelin A in the supernatant sample was in the range of 0.02 to 0.10 M.

Processing Description

Peaks were identified by a combination of their characteristic masses, retention times, and their UV-vis absorbance using MassHunter (Agilent).

BCO-DMO Data Manager Processing Notes:
* Imported data table from file "Azotobacter_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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Related Publications

Baars, O., Zhang, X., Morel, F. M. M., & Seyedsayamdost, M. R. (2016). The Siderophore Metabolome of Azotobacter vinelandii. Applied and Environmental Microbiology, 82(1), 27–39. doi:10.1128/aem.03160-15 https://doi.org/10.1128/AEM.03160-15
Methods

Zhang, X., Baars, O., & Morel, F. M. M. (2019). Genetic, structural, and functional diversity of low and high-affinity siderophores in strains of nitrogen fixingAzotobacter chroococcum. Metallomics, 11(1), 201–212. doi:10.1039/c8mt00236c
Results

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Parameters

Parameter	Description	Units
Time_days	Sampling time from start of incubation (decimal days).	days
Organism	Species and strain of Azotobacter bacterium used in the culture (e.g. Achroococcum_8003).	unitless
Condition	Growth conditions were one of the following three: precipitated amorphous Fe oxides / 100 mM EDTA and 5 mM FeCl3 / 100 mM EDTA and 0.1 mM FeCl3	unitless
vibrioferrin	Concentration of the siderophore vibrioferrin.	micromoles per liter (umol/L, uM)
amphibactins	Summed concentration of a suite of amphibactin siderophores.	micromoles per liter (umol/L, uM)
crochelin_A	Concentration of the siderophore crochelin A.	micromoles per liter (umol/L, uM)
catechol_siderophores	Summed concentration of the siderphores protochelin, azotochelin, aminochelin, and 2,3-dihydroxybenzoic acid.	micromoles per liter (umol/L, uM)
azotobactins	Summed concentration of various azotobactin type siderophores described in Baars, Oliver, et al. (2006).	micromoles per liter (umol/L, uM)
OD620	Optical density of the culture measured at 620 nm	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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