Siderophore concentrations found in supernatants of Rhodopseudomonas palustris str. CGA009 grown under different aerobic and anaerobic conditions from laboratory experiments in 2016

Website: https://www.bco-dmo.org/dataset/861145
Data Type: experimental
Version: 1
Version Date: 2021-09-21

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

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Abstract
Siderophore concentrations found in supernatants of Rhodopseudomonas palustris str. CGA009 grown under different aerobic and anaerobic conditions from laboratory experiments in 2016. These data were published in Baars et al. (2018, Fig. 3).

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Coverage

Temporal Extent: 2016

Methods & Sampling

Sampling and analytical procedures:

R. palustris strain CGA009 was grown in batch culture at room temperature in the presence or absence of added iron and molybdate under (i) aerobic chemoheterotrophic growth supplemented with 2 mM ammonium, (ii) photoheterotrophic nitrogen-fixing growth under anaerobic conditions and (iii) photoheterotrophic nitrogen-fixing under anaerobic conditions with added NaS and cysteine as reductants. Aerobic cultures (30 ml) in Nunc flasks (75 cm2) were shaken at 45 rpm. Before the start of the incubations, bacteria were adjusted for >12 generations (> 2 transfers into fresh media) to the growth conditions. Photoheterotrophic anaerobic cultures (10 ml) were grown with a constant light source in Baltch-type glass tubes (25 ml volume) with a nitrogen

headspace, sealed with a butyl rubber stopper. For all anaerobic incubations, bacteria were grown for >30 generations (5 transfers into fresh media) in media supplemented with Fe but without Mo. This was necessary to dilute Mo originally present in the medium to levels low enough for a clear measurable Mo growth limitation. All incubations were performed in duplicate. Bacterial growth was monitored spectrophotometrically as optical density at 660 nm. To confirm the absence of oxygen in rhodopetrobactin producing cultures, samples were taken occasionally in a glovebox (COY Vinyl) for measurement of dissolved oxygen with an oxygen probe (Hach HQ40d) and redox potential with an ORP probe (Hana HI-98120) or the redox indicator resazurine. Dissolved oxygen concentrations were always below the detection limit of the oxygen sensor (< 0.02 mg/l). Redox potential measurements with the ORP probe in anaerobic treatments were <90 mV and <150 mV vs. NHE in media without and with added reductants. Reductant additions to anaerobic media clarified the redox indicator resazurine indicating a redox potential <110 mV and had a noticeable H2S smell.

Bacterial incubation samples (1 ml) were collected throughout growth, filtered through 0.2 m syringe filters (Millipore MILLEX GP 0.22 m) and the supernatants were stored at 2208C until analysis. Quantification of rhodopetrobactins was performed on a single quadrupole LC-MS system (Agilent 6120), equipped with a diode array detector. Prior to analysis, the samples were acidified with 0.1% acetic acid and 0.1% formic acid. Samples (100 L) were injected onto a C18 column (Agilent Eclipse Plus C18, 3.5 m, 4.6x100 mm) equipped with a matching guard column. The separation proceeded with A and B solutions (solution A: water, 0.1% formic acid, 0.1% acetic acid; solution B: acetonitrile, 0.1% formic acid, 0.1% acetic acid) over 30 min, at a flow rate of 0.8 ml/min. Using a 6-port valve, the column outflow was diverted to waste for the first 5.25 min ensuring that the sample was desalted before introduction into the mass spectrometer. For quantification, UV/Vis traces were extracted at 294 nm, and peak areas corresponding to the elution of rhodopetrobactin A and B were determined using MassHunter software (Agilent). Concentrations were determined with standard solutions of 3,4-dihydroxybenzoic acid and isolated rhodopetrobactins. The detection limit for rhodopetrobactins was approximately 0.1 M.

Iron concentrations in the supernatants were measured by inductively coupled plasma-mass spectrometry (Thermo iCAP-Q in KED mode) after acidification (10% HNO3) and dilution (1:5). The detection limit for iron (^{56}Fe) was approximately 0.065 M.

Data 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 "Rpalustris_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
rpalustris_conc.csv (Comma Separated Values (.csv), 2.97 KB) MD5:15d4ccc6bf7b6432ff8ae8d05cc4add2 Primary data file for dataset ID 861145

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Related Publications

Baars, O., Morel, F. M. M., & Zhang, X. (2018). The purple non-sulfur bacterium Rhodopseudomonas palustris produces novel petrobactin-related siderophores under aerobic and anaerobic conditions. Environmental Microbiology, 20(5), 1667–1676. doi:10.1111/1462-2920.14078
Results

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Parameters

Parameter	Description	Units
Time_days	Sampling time from start of incubation (decimal days).	days
Condition	Growth conditions were one of the following: (a) Aerobic and chemoheterotrophic | (b) Anaerobic photoheterotrophic N2-fixing | (c) Anaerobic photoheterotrophic N2-fixing with a chemically reduced medium using Na2S and cysteine. Each of the above conditions was micronutrient replete (+Fe/+Mo) or limited for either Fe (-Fe/+Mo), Mo (+Fe/-Mo) or both micronutrients (-Fe/-Mo).	unitless
rhodopetrobactin_A	Concentration of the siderophore rhodopetrobactin A.	micromoles per liter (umol/L, uM)
rhodopetrobactin_B	Concentration of the siderophore rhodopetrobactin B.	micromoles per liter (umol/L, uM)
OD660	Optical density of the culture measured at 660 nm.	micromoles per liter (umol/L, uM)
dissolvedFe	Concentration dissolved iron	micromoles per liter (umol/L, uM)
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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