Halomethane concentrations in cell culture

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Data Type: experimental

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Project

» The role of organic and metal cofactors on the biogenic synthesis of halogenated volatile hydrocarbons (Volatile_Hydrocarbons)

Contributors	Affiliation	Role
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Abstract

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Dataset Description

Six taxonomically diverse strains of marine bacteria: *Alphaproteobacteria* (DFL12 and MED193), *Gammaproteobacteria* (AND4) and *Bacteroidetes* (MED134, MED152 and MED217) were grown in culture. Cultures were grown in 120 mL ZoBell marine broth medium in 250 mL glass vials (ca. 120mL headspace) at 22°C on a shaker at 10rpm and full light conditions using an artificial light source maintained at approximately 125 µmol photons m-2 s-1 until they reached stationary phase, which ranged from ca. 8 to 100 hours. Cultures reached high cell densities, typically on the order of 107 - 108 cells mL-1 during late log growth phase, and one final "late" time-point was taken 24 hours after the last experimental replicates to assess behavior in stationary phase. Duplicate culture vials were killed by acidification to ca. pH 2.0 with HCl (0.5mL 3mol L-1 solution for 125mL culture volume) HCl and refrigerated prior to analysis where they are stable for up to two weeks (EPA 1986). Samples were analyzed for dissolved halocarbon concentrations using a gas chromatography (GC) method adapted from Schall and Heumann (1993) and quantified relative to an internal standard. For cell abundance and volume determination, duplicate samples were fixed with 10% formalin (4% formaldehyde), stained with acridine orange (Hobbie et al. 1977), filtered onto pre-blackened filters and counted with epifluorescence microscopy. Purge-and-trap capillary column gas chromatography with electron capture detection (GC-ECD) was employed for dissolved halocarbon analysis (Schall and Heumann 1993). 25mL media samples were purged with ultra-high purity He for 45min at a flow rate of 60mL min-1 through an in-line K2CO3 drying tube and onto a liquid nitrogen trap. The purge vessel is rinsed with methanol and the drying trap replaced with 0.75g fresh K2CO3 between individual analyses. Cryo-concentrated samples were introduced into an Agilent 7890A GC by means of a splitless injection with sweep pressure at 50psi for 1.5min returning to analytical column pressure of 18psi 2.5min after injection. Inlet temperature was set to 60°C to facilitate cryo-focusing on the column. Initial oven temperature was 40°C for 10min increasing to 120°C by 4°C min-1 and held there for another 2min. Temperature was then ramped to a final 240°C at a rate of 5°C min-1 and held for 20min Calibration was carried out using 20µL of 0.5µg/L of tribromochloromethane as an internal standard (Gonzalez-Gago et al. 2007).

Acquisition Description

Peak Simple model 302 Integration using Peak 393 software
Excel 2016

Processing Description

BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- replaced spaces and - with underscores
- blank values replaced with no data value 'nd'

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Parameters

Parameter	Description	Units
Strain	Strain of marine bacteria	unitless
Species	Species	unitless
Clade	Clade	unitless
Time_elapsed_hours	Incubation duration; originally Time (hours)	hours
cells_per_ml	Cell concentration; originally cellsmL-1	cells per milliliter
CH3Br	bromomethane concentration	picomoles per liter
CH3I	iodomethane concentration	picomoles per liter
CH3Cl	chloromethane concentration	picomoles per liter
CHBr3	bromoform, tribromomethane concentration	picomoles per liter

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Instruments

Dataset-specific Instrument Name	Agilent 7890A Gas Chromatograph with electron capture detector
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Column- Restek Rtx-502.2 (60m, 0.32mm ID, 1.8µm df)
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

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Project Information

The role of organic and metal cofactors on the biogenic synthesis of halogenated volatile hydrocarbons (Volatile_Hydrocarbons)

Volatile halogenated hydrocarbon gases, in this case halomethanes, are produced naturally by organisms in the ocean; which then serves as a source of these biogenic gases to the atmosphere. Their chemical reactions in the atmosphere are very similar to those of anthropogenic chlorofluorocarbons (CFCs). While CFCs are well-studied because they consume the ozone in the upper atmosphere that shields the earth from harmful ultraviolet radiation, halomethanes have been largely neglected, even though they currently account for 25% of the ozone depletion. As anthropogenic CFC levels steadily decline, however, halomethanes are predicted to account for 50% of ozone depletion by 2050. Based on limited study thus far, marine halomethane production has been ascribed mainly to phytoplankton and macro algae. This project will build on new and compelling data that suggests marine heterotrophic bacteria could also be major producers of halomethanes. The data produced here will provide the critical evaluation required to address discrepancies in global halomethane budgets which currently are out of balance due to an unknown source to the atmosphere, evaluating the hypothesis that marine heterotrophic bacteria can supply this missing source. Concerns over the stability of the earth's stratospheric ozone layer make this valuable and necessary research with added value of providing support for engaged undergraduate, graduate, and postdoctoral education at the University of Southern California. Past research on the production of marine halomethanes has focused on phytoplankton and macro algae, while potential bacterial contributions to the processe have been neglected. This research proposes to study the role of marine heterotrophic bacteria on the production of halomethanes. It has been noted in past studies that there are discrepancies in the global atmospheric halomethane budget, and it is possible this is due to a large missing bacterial source. Additionally, this research will evaluate the potential importance of vitamin B12, methionine, and vanadium cofactors on the synthesis of halomethanes in bacteria. A large portion of marine bacteria cannot synthesize methylation co-enzymes, and therefore, would require available B12, methionine, and vanadium from external sources to complete the methylation step. This study will also measure concentrations of halomethanes, B12, methionine, and vanadium in upwelling regions as well as at a long-term time series site in order to put constraints on the variability of halomethanes concentrations for use in global linked air-sea models.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1559276

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