Metabolic potential for heterotrophic utilization of a large array of organics by coccolithophores determined through experiments at Bigelow Laboratory for Ocean Sciences using BioLog Eco-plates

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Project
» Coccolithophore Mixotrophy (Cocco-Mix)

Contributors	Affiliation	Role
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Abstract
This dataset includes results from an experiment determining the metabolic potential for heterotrophic utilization of a large array of organics by coccolithophores. Experiments used the BioLog Eco-plates (BioLog, Haywood, CA, U.S.A.) and were conducted at Bigelow Laboratory for Ocean Sciences, East Boothbay, ME.

Table of Contents

Coverage

Spatial Extent: **N**:59.5 **E**:47.8333 **S**:-13.5833 **W**:-113.56
Temporal Extent: 1951-01-01 - 2013-11-20

Acquisition Description

BioLog Eco-plates contained 96-wells, prefilled with: (1) a colorless tetrazolium dye that reduces to a violet formazin if the substrate is oxidized, (2) triplicates of 31 different organic compounds in equimolar quantities, and (3) triplicate water blanks as a control for airborne bacterial contamination. We used a single coccolithophore strain in each plate. We started each experiment by inoculating a 96-well plate with 100 µL of log-phase cell suspension from each log phase culture. We carried out all inoculations under subdued light and all incubations in complete darkness. At time-zero (T0), 24 h, 48 h, and 72 h, we

measured the reduced violet tetrazolium dye absorption on a FilterMax F5 multimode plate reader (Molecular Devices, LLC, San Jose, CA, U.S.A.) for absorption at 595 nm. We interpreted the increasing optical density at 595 nm as heterotrophic metabolism of the organic compound by the coccolithophore cultures. (Note: For two strains, CCMP298 and CCMP 3337, T72 was not performed, but the measurement was instead taken at T120.)

Several limitations to the BioLog Eco-plates should be noted (Stefanowicz 2006). One limitation is that the technique assumes that the uptake of each substrate is independent from any other (i.e., two substrates are not used in a synergistic fashion). The technique also assumes the concentrations of the substrates in each well of the microtiter plate are optimal for the organism in question, rather than too high (i.e., inhibitory) or low (limiting) based on the organism's specific uptake kinetic parameters. Therefore, we could not make any inferences on the uptake kinetics of DOC utilization using the microtiter plates. Furthermore, ions in seawater (specifically Ca++) can cause false positives in BioLog Eco-plates (Pierce et al. 2014). It is critical to lower the Ca++ concentration from 10 mmol L^{-1} (normal concentration in seawater) to ~ 2.5 mmol L^{-1} in order to eliminate false positives (Pierce et al. 2014). The BioLog Company suggests doing this with chelators. An alternative method was suggested by Tuchman et al. (2006) in which the cultures were centrifuged and pelleted to separate them from the nutrient-rich media, which might contain other growth-inducing substrates. Then the pellet should be resuspended in nutrient-free saline solution. We followed these latter recommendations and resuspended the coccolithophores in ASW with 2.5 mmol L^{-1} calcium.

Processing Description

Data Processing:
The potential utilization of each organic compound was expressed as the average compound color development (ACCD). We averaged the absorption observed at five points across the center of each well at each time point and subtracted the average water control well. We then calculated the mean absorbance of replicate wells for each compound. The ACCD was computed as the percentage of the absorbance of each compound over the sum of absorbance of all compounds on a plate.

BCO-DMO Processing:
- concatenated data from separate Excel sheets into one dataset;
- joined the strain information from original file "CCMP_cocco_strains.xlsx" to the rest of the dataset, matching on the Strain_code field;
- renamed fields to conform with BCO-DMO naming conventions;
- replaced 'n.d.' with 'nd' (no data);
- converted dates to YYYY-MM-DD format;
- removed directionals from latitude and longitude column to allow for column to be typed as numeric;
- removed comma in D,L-α-Glycerol-Phosphate in Substrate column to allow for download as a .csv;
- replaced spaces in Species column and Collection_Site_Sea column with underscores to allow for sorting;
- rounded data as requested by data provider.

[table of contents | back to top]

Related Publications

Godrijan, J., Drapeau, D., & Balch, W. M. (2020). Mixotrophic uptake of organic compounds by coccolithophores. Limnology and Oceanography, 65(6), 1410–1421. doi:10.1002/lno.11396
Results

Pierce, M. L., Ward, J. E., & Dobbs, F. C. (2014). False positives in Biolog EcoPlatesTM and MT2 MicroPlatesTM caused by calcium. Journal of Microbiological Methods, 97, 20–24. doi:10.1016/j.mimet.2013.12.002
Methods

Stefanowicz, A. 2006. The Biolog plates technique as a tool in ecological studies of microbial communities. Pol. J. Environ. Stud. 15: 669–676. https://isbnsearch.org/isbn/1230-1485
Methods

Tuchman, N. C., Schollett, M. A., Rier, S. T., & Geddes, P. (2006). Differential Heterotrophic Utilization of Organic Compounds by Diatoms and Bacteria under Light and Dark Conditions. Hydrobiologia, 561(1), 167–177. doi:10.1007/s10750-005-1612-4
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Strain_code	Strain code (CCMP) from the National Center for Marine Algae and Microbiota (NCMA)	unitless
Species	Species name	unitless
Isolated_Date	Strain isolation date; format: YYYY-MM-DD	unitless
Deposit_Date	Strain deposit date; format: YYYY-MM-DD	unitless
Collection_Site_Lat	Latitude of strain collection site; positive values = North	decimal degrees North
Collection_Site_Long	Longitude of strain collection site; positive values = East	decimal degrees East
Collection_Site_Sea	Body of water where strain was collected	unitless
Substrate	Substrate	unitless
T0_AVG	Average absorption at time-zero (T0)	unitless
T0_SD	Standard deviation of absorption at time-zero (T0)	unitless
T24_AVG	Average absorption at 24 hours	unitless
T24_SD	Standard deviation of absorption at 24 hours	unitless
T48_AVG	Average absorption at 48 hours	unitless
T48_SD	Standard deviation of absorption at 48 hours	unitless
T72_AVG	Average absorption at 72 hours	unitless
T72_SD	Standard deviation of absorption at 72 hours	unitless
T120_AVG	Average absorption at 120 hours	unitless
T120_SD	Standard deviation of absorption at 120 hours	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	FilterMax F5 multimode plate reader
Generic Instrument Name	plate reader
Dataset-specific Description	FilterMax F5 multimode plate reader (Molecular Devices, LLC, San Jose, CA, U.S.A.).
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

[table of contents | back to top]

Project Information

Coccolithophore Mixotrophy (Cocco-Mix)

Coverage: Partially lab-based, with field sites in Gulf of Maine and NW Atlantic between the Gulf of Maine and Bermuda

Coccolithophores are unicellular haptophyte algae generally thought of as photoautotrophs. They are covered with scales or "coccoliths" (made of calcium carbonate (particulate inorganic carbon, PIC)). Recent observations suggest that globally, haptophytes contribute more biomass than ubiquitous *Prochlorococcus* and *Synechococcus*. Coccolithophores can affect the draw-down of atmospheric CO2 and are involved in two fundamental "pump paradigms": (1) The alkalinity pump (also known as the carbonate, PIC, or CaCO3 pump) lowers total alkalinity (TA) and dissolved inorganic carbon (DIC) in the euphotic zone during calcification, and increases upper ocean and atmospheric CO2. Coccoliths eventually sink below the ocean’s lysocline (the depth where calcium carbonate dissolves), where they release the bicarbonate back into deep water. Thus, they essentially "pump" bicarbonate alkalinity from surface to benthic waters, where it remains isolated in the deep sea for thousands of years. (2) The biological pump in which the ballasting effect of the heavy coccoliths on sinking particulate organic carbon (POC) increases the magnitude of the soft tissue (POC) pump, which ultimately decreases surface CO2. The soft-tissue and alkalinity pumps reinforce each other in maintaining a vertical gradient in DIC but they oppose each other in terms of the air-sea exchange of CO2. Thus, the net effect of coccolithophores on atmospheric CO2 depends on the balance of their CO2-raising effect associated with the alkalinity pump and their CO2-lowering effect associated with the soft-tissue biological pump. It is virtually always assumed that the PIC found in coccoliths originates exclusively from DIC, not dissolved organic carbon (DOC). However, there is an increasing body of evidence that coccolithophores are mixotrophic (defined as a combination of growth fueled by autotrophy, uptake of DOC and phagotrophy of small particles (POC). This proposal is to describe

the potential uptake and assimilation of an array of DOC compounds in the sea, the kinetics of their uptake and potential incorporation of organic carbon by coccolithophores into PIC coccoliths (which could significantly alter the alkalinity pump paradigm since calcite production in the surface ocean would not be at the expense of bicarbonate).

This work is fundamentally directed at quantifying coccolithophore mixotrophy in lab of technological advances to address this issue, all of which we will apply in this work. We will: (a) screen axenic coccolithophore cultures for the uptake and oxidation of a large array of potential DOC substrates, (b) perform radiolabel-uptake experiments with these molecules using high-specific activity substrates in order to provide the basic kinetic response at environmentally-realistic concentrations, (c) measure radio-labelled carbon fixed into organic tissue, separate from that fixed into PIC, (d) sort 14C-labelled coccolithophores free of the other free-living phytoplankton and bacteria using flow cytometry and e) distinguish the modes of nutrition in these sorted coccolithophore cells. This work will advance the state of knowledge of coccolithophore mixotrophy in the marine environment and address the balance of carbon that coccolithophores derived from autotrophic versus heterotrophic sources.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1635748

[table of contents | back to top]