LC-MS/MS sequenced proteins extracted from Stylophora pistillata skeleton; analyzed in the Falkowski lab at Rutgers from 2010-2014 (CROA project)

Website: https://www.bco-dmo.org/dataset/537964
Version: 13 Nov 2014
Version Date: 2014-11-13

Project
» The Molecular Basis of Ocean Acidification Effects on Calcification in Zooxanthellate Corals (CROA)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
Falkowski, Paul G.	Rutgers University (Rutgers IMCS)	Principal Investigator, Contact
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Dataset Description

This dataset includes information presented in Supplemental Table S2 from Drake et al. 2013:
Table S2. Putative homologous proteins from other mineralizers or related organisms. The most similar predicted protein sequence from each comparison organism is given. Lack of a similar protein sequence for a given species is noted as "–".
See the original Excel file for the complete sequences of the most similar predicted protein sequence from each comparison organism.

Refer to the following publication for more information:
Drake, J. L., T. Mass, L. Haramaty, E. Zelzion, D. Bhattacharya & P. G. Falkowski. 2013. Proteomic analysis of skeletal organic matrix from the stony coral *Stylophora pistillata*. Proceedings of the National Academy of Sciences 110(10): 3788-3793. doi: 10.1073/pnas.1301419110

Also refer to:
Figure S2. Predicted amino acid sequences of 36 S. pistillata proteins (PDF). Peptides detected by LC-MS/MS after tryptic digestion are in bold and after proteinaseK digestion are underlined. Translations of internal sequences confirmed by PCR amplification of *S. pistillata* cDNA using gene-specific primers are highlighted in gray. Discrepancies between the predicted sequence and that determined by translation of PCR product are in

red. The secretion signal peptide of P12, is crossed out over the portion that is predicted to be cleaved before secretion.
Table S1. SOM protein primer sets (PDF). Gene-specific primers used to confirm the DNA and cDNA sequences of selected SOM proteins.

Methods & Sampling

Methodology described in Drake et al. 2013:
SOM Extraction: *S. pistillata* skeletons were soaked for 4 hours in 3% (wt/vol) sodium hypochlorite, copiously rinsed in deionized water, and dried overnight at 60 degrees C. Dried skeletons were ground to a fine powder with an agate mortar and pestle and again bleached, rinsed, and dried. The skeletal powder was decalcified in 1 N HCl at room temperature while shaking. HCl was added gradually so that the solution reached neutral pH within 30 min of acid addition; more HCl was only added if skeleton powder remained after 30 min. pH of the decalcification solution was brought to neutral with 1 M NaOH. Water-soluble and -insoluble organic fractions were separated by centrifugation and analyzed separately. Trichloroacetic acid (TCA)-acetone precipitations were used to clean and precipitate proteins from the decalcification solution. Briefly, one volume of 60% (wt/vol) TCA was added to five volumes soluble SOM samples and 1 mL 60% (wt/vol) TCA was added to insoluble SOM pellets. Both fractions were incubated at 4 degrees C overnight, centrifuged at 10,000 x g at 4 degrees C for 30 min, washed twice with ice-cold 90% (vol/vol) acetone at 4 degrees C for 15 min, and centrifuged at 10,000 x g at 4 degrees C for 30 min. Additionally, SOM proteins were enzymatically deglycosylated with O-glycosidase, N-glycosidase F, sialidase, B1-4 galactosidase, and B-N-acetylglucosaminidase in a deglycosylation mix per manufacturer instructions (New England BioLabs).

Protein Separation and Characterization: SOM proteins were separated by SDS/PAGE and bands were visualized by silver staining (Pierce silver stain for mass spectrometry) and Periodic acid-Schiff staining (Pierce glycoprotein staining kit). Smearing of proteins in gels precluded extraction of individual bands for sequencing.

Proteomics: SOM complexes were digested either by trypsin or proteinase K, and masses and charges of the digested peptides were analyzed on a Thermo LTQ-Orbitrap-Velos ETD mass spectrometer with Dionex U-3000 Rapid Separation nano LC system. The LC-MS/MS data were searched using predicted gene models from *S. pistillata* by X! Tandem using an in-house version of the Global Proteome Machine (GPM USB; Beavis Informatics) with carbamidoethyl on cysteine as a fixed modification and oxidation of methionine and tryptophan as a variable modification. Spectra were also analyzed against a suite of potential microbial genomes to exclude possible microbial contamination of the dry skeleton. Data for LC-MS/MS sequenced proteins have been deposited in GenBank.

Gene Confirmation: Internal sequences of predicted genes were confirmed in DNA and cDNA by PCR using gene-specific primers (Table S2). Holobiont DNA and cDNA were prepared as previously described from *S. pistillata* colonies maintained in in-house aquaria. All PCR tubes contained 0.25-ug template, 0.2 mM dNTPs, 1 x High Fidelity reaction buffer, 0.5 uM of each primer, and 0.04 units uL−1 of Phusion polymerase (New England BioLabs) in a 25-uL reaction volume. Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems) at 35 cycles of 98 degrees C for 10 s, primer-specific annealing temperature for 30 s, and 72 degrees C for 30–180 s. PCR products were sequenced by GENEWIZ.

Bioinformatics: LC-MS/MS results were filtered to remove hits from standard contamination (common Repository of Adventitious Proteins, or cRAP, database). A nonredundant list of all proteins detected with e-values ≤10−10 was used for blast analysis against NCBI and to query a database we created that contains translated sequences from *Homo sapiens*, *Thalassiosira pseudonana* [diatom], *Nematostella vectensis* [anemone], *Strongylocentrotus purpuratus* [urchin], *E. huxleyi* CCMP1516 (coccolithophore; draft genome), and *A. digitifera* [hard coral] genomes; a transcriptome from *P. damicornis* [hard coral]; and expressed sequence tag (EST) libraries from *Favia sp.* [hard coral], *Reticulomyxa filosa* [foraminiferan], and *P. maxima* [oyster]. *N. vectensis* and *R. filosa* do not biomineralize; all other comparison species produce calcium- or silica-based minerals. Predicted proteins from the comparison species with similarities greater than 35% and e-values ≤10−10 were retained for further analysis. For CARP subfamily homologs, predicted proteins in comparison species were combined if they closely mimicked matched *S. pistillata* CARPs. These combinations are noted in protein names when they are presented in the multiple sequence alignment. Residues whose conservation suggests a functional role were predicted in ConSurf using CARP4 as the query sequence. Structures of selected proteins were predicted using both I-TASSER and Phyre2. We used these two programs to obtain a consensus in structure matching, particularly in the case of one S. pistillata protein that showed no similarity to proteins in the NCBI and contained no known domains. Glycosylation sites were predicted using the EnsembleGly server at the AIRL at Iowa State University. Images of predicted structures were generated in

MacPyMOL v1.3r1 (Schrodinger).

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Data Files

File
SI_T1_copy.csv (Comma Separated Values (.csv), 4.73 KB) MD5:5d74feaa680377d21c0ef0fad0e2a709 Primary data file for dataset ID 537964

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Parameters

Parameter	Description	Units
transcript	Name/identifier of the protein.	dimesnsionless
gene	Name/identifier of the gene; the code number used in the S. pistillata gene prediction model.	dimesnsionless
accession_number	GenBank accession number.	dimesnsionless
H2O_soluble	Whether or not the protein candidate was in the water-soluble fraction (+ = yes; - = no)	+/- (yes/no)
H2O_insoluble	Whether or not the protein candidate was in the water-insoluble fraction (+ = yes; - = no)	+/- (yes/no)
observed_in_glycosylated_samples	Whether or not the protein candidate was observed in glycosylated samples (+ = yes; - = no)	+/- (yes/no)
better_detection_after_deglycosylation	Whether or not the protein candidate was better detected after deglycosylation (+ = yes; - = no)	+/- (yes/no)
MS_hit_w_trypsin	Whether or not the protein candidate was observed in tryptic digestion (+ = yes; - = no)	+/- (yes/no)
MS_hit_w_proteinase_K	Whether or not the protein candidate was observed after proteinase K digestion (+ = yes; - = no)	+/- (yes/no)
accession_num_link	Hyperlink to GenBank for the specific accession number.	dimesnsionless

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Instruments

Dataset-specific Instrument Name	Thermo LTQ-Orbitrap-Velos ETD mass spectrometer
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	SOM complexes were digested either by trypsin or proteinase K, and masses and charges of the digested peptides were analyzed on a Thermo LTQ-Orbitrap-Velos ETD mass spectrometer with Dionex U-3000 Rapid Separation nano LC system.
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.

Dataset-specific Instrument Name	Veriti Thermal Cycler (Applied Biosystems)
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Amplifications were performed in a Veriti Thermal Cycler (Applied Biosystems) at 35 cycles of 98 degrees C for 10 s, primer-specific annealing temperature for 30 s, and 72 degrees C for 30–180 s. PCR products were sequenced by GENEWIZ.
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

lab_Falkowski

Website	https://www.bco-dmo.org/deployment/537725
Platform	Rutgers_New_Brunswick
Start Date	2010-09-01
End Date	2014-08-01
Description	Laboratory-based research for the project "The Molecular Basis of Ocean Acidification Effects on Calcification in Zooxanthellate Corals" were conducted at Dr. Falkowski's lab at the Rutgers New Brunswick campus: 71 Dudley Road New Brunswick, NJ 08901

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Project Information

The Molecular Basis of Ocean Acidification Effects on Calcification in Zooxanthellate Corals (CROA)

Website: http://corals.marine.rutgers.edu/

Coverage: laboratory-based in New Brunswick, NJ

From the NSF award abstract:

Ocean acidification (the decrease in seawater pH) is driven by the increase in atmospheric CO2. This is expected to have a dramatic effect on organisms that precipitate calcium carbonate. Coral reefs are formed and maintained by calcifying organisms, particularly reef-building corals. Current predictions are that coral species will be negatively impacted; however the limited number of available measurements exhibit significant variability for reasons that are not understood. This is critically important as coral reef ecosystems hold significant cultural and economic values both nationally and internationally. This program is therefore focused on the molecular basis for calcification in corals in order to understand how corals will respond to ocean acidification in the next century. Rutgers University has a state-of-art coral culture facility that will be used to simulate future ocean conditions. The work will utilize a unique set of coral tissue cultures that will allow scientists to assess the cellular biology that underlies the responses of corals to ocean acidification. The laboratory measurements will also determine how geochemical signatures of corals are affected by varying environmental conditions. These results are important because coral geochemical signatures are used to understand how corals have responded to changes in the ocean pH in the historical past. The project will be conducted by a research team at Rutgers, in collaboration with scientists in Taiwan and Israel.

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Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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Funding

Funding Source	Award
NSF Emerging Frontiers Division (NSF EF)	EF-1041143

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