Partial SSU rRNA genes of bacteria and archaea from reef seawater samples produced using 515F/806R and 515F/806RB primers from the Bermuda, Red Sea, and Federated States of Micronesia in 2013 (Coral Microbial Relationships project)

Website: https://www.bco-dmo.org/dataset/565723
Version: 2015-09-10

Project
» Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

Contributors	Affiliation	Role
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Table of Contents

Dataset Description

Relevant Reference:

Apprill A, McNally S, Parsons R, Weber L (2015) Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol 75:129-137. doi: 10.3354/ame01753

Methods & Sampling

Seawater sample collection

Nucleic acids were collected from surface waters along the coastline of the southeastern Red Sea and from coral reefs surrounding the islands and atolls in the Federated States of Micronesia (Supplementary Table 1). Samples from an aquaria-based experiment housed at the Bermuda Institute of Ocean Sciences (BIOS) were also utilized in this study. Seawater from Ferry Reach, Bermuda (32.37035°N, 64.69545°W) was sampled from a direct inflow line. In addition, this water was also held in separate aerated 30 liter aquaria (similar to de Putron et al. 2011) where it was sampled for nucleic acids (500 ml) and fluorescence *in situ* hybridization (FISH) analyses (50 ml) over the course of 12 days.

DNA analysis

Seawater collected from the Red Sea (20 liters) and Micronesia (2 liters) was filtered onto 142 mm, 0.22 µm Durapore membrane filters (Millipore, Boston, MA, USA) and 25 mm 0.2 µm polyethersulfone membranes (Supor, Pall, East Hills, NY, USA), respectively, and immediately frozen in liquid nitrogen. Total genomic DNA was extracted from these samples using previously reported methods (Santoro et al. 2010). Similarly, microbial biomass originating from the BIOS seawater inflow line and aquaria seawater was concentrated onto 0.2 µm polyethersulfone membranes (Supor) using a 47 mm support filter and a gelman rig under gentle vacuum (~100mm Hg). Each filter was stored in 1 ml of sterile sucrose lysis buffer (20mM EDTA, 400mM NaCl, 0.75M sucrose, 50mM Tris.HCl) at –80°C. DNA was extracted using the phenol-chloroform method

(Giovannoni et al. 1990). Primers targeting the V4 region of the SSU rRNA gene, 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al. 2011), were utilized for PCR amplification using unique barcoded primer combinations for each sample. In addition, the same DNA samples were amplified separately with the 515F primer and a modified 806RB primer using the identical barcoding approach. This modified reverse primer replaces the 'H' degeneracy in the original 806R primer with a 'N', and is designed to enhance SAR11 targets (revised primer 806RB, 5'-GGACTACNVGGGTWTCTAAT-3'). The primers were designed after Kozich and colleagues (2013) and were each equipped with a unique 8-bp barcode, 10-bp pad and 2-bp link that followed the above-mentioned primers (see Supplementary Materials and Methods). Triplicate 25 µl PCR reactions were conducted per sample and each reaction contained 1.25 U of GoTaq Flexi DNA Polymerase (Promega Cooperation, Madison, WI, U.S.A.), 5X Colorless GoTaq Flexi Buffer, 2.5 mM MgCl2, 200 uM dNTP mix (Promega Cooperation, Madison, WI, U.S.A.), 200 nM of each barcoded primer, and 1 - 4 ng of genomic template. The reaction conditions consisted of an initial denaturation step at 95ºC for 2 min, followed by 27-38 cycles of 95ºC for 20 s, 55ºC for 15 s, and 72ºC for 5 min, concluding with an extension step at 72ºC for 10 min. The reactions were carried out on a Bio-Rad thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Reaction products (5 µl) were screened on a 1% agarose/TBE gel. The HyperLadder 50bp DNA ladder (generally 5ng µl-1) (Bioline, London, UK) was used to confirm appropriate amplicon size. The number of PCR cycles varied between samples in order to produce similar, minimal yields, but each sample was subjected to nearly identical PCR cycles with both primer sets. The three replicate reactions were pooled and subsequently purified using the QIAquick Purification Kit (Qiagen, Valencia, CA, USA), and quantified using the Qubit 2.0 Fluorometer with the dsDNA High Sensitivity Assay (Life Technologies, Grand Island, NY USA). For each primer set, barcoded amplicons were pooled into equimolar ratios. These amplicon pools were then shipped to the University of Illinois W.M. Keck Center for Comparative and Functional Genomics where they were used for construction of two separate libraries which were subsequently sequenced using 2x250bp paired-end MiSeq (Illumina, San Diego, CA, USA), as detailed previously (similar to Kozich et al. 2013). Control samples included sterile water (negative controls) in which PCR did not yield any detectable amplification with either primer set. A mock community sample (positive control, obtained through BEI Resources, NIAID and NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (even, low concentration), v5.1L, HM-782D) was amplified with the 515F/806RB primers and sequenced to assess amplification and sequencing error rate.

Microbial abundances and FISH

To determine microbial abundances from the BIOS inflow and aquaria samples, the seawater was fixed to 10% formalin and stored at -80°C. Upon analysis, the samples were thawed and filtered onto 0.2µm filters pre-stained with Irgalan black (0.2g in 2% acetic acid) under gentle vacuum (~100mm Hg) and post-stained with 0, 6-diamidino-2-phenyl dihydrochloride (5µg ml-1, DAPI, SIGMA-Aldrich, St. Louis, MO, USA) (Porter and Feig 1980). Slides were then enumerated using an AX70 epifluorescent microscope (Olympus, Tokyo, Japan) under ultraviolet excitation at 100x magnification as previously described (Parsons et al. 2014). At least 500 cells per filter (12 fields) were counted.

Fluorescence in situ hybridization (FISH) was used to quantify the abundance of SAR11 in the BIOS aquaria samples using a probe suite (152R-Cy3, 441R-Cy3, 542R-Cy3, 732R-Cy3) and was conducted as previously described (Morris et al. 2002, Parsons et al. 2012). Image analysis coupled with epifluorescence microscopy was used to process FISH slides excited with Cy3 (550nm) and UV wavelengths. The image capturing was performed using a Retiga Exi CCD digital camera with QCapture software version 2.0 (QImaging, Burnaby, BC, Canada) and processed with Image Pro software (version 7.0; Media Cybernetics, Bethesda, MD, USA) as previously described (Parsons et al. 2014). SAR11 percentages were calculated as SAR11 FISH abundances compared to total cellular abundances.

References:

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516-4522.

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Kozich, J. J., S. L. Westcott, N. T. Baxter, S. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79:5112-5120.

Morris, R. M., M. S. Rappé, S. A. Connon, K. L. Vergin, W. A. Siebold, C. A. Carlson, and S. J. Giovannoni. 2002. SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420:806-810.

Parsons, R. J., M. Breitbart, M. W. Lomas, and C. A. Carlson. 2012. Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea. ISME J 6:273-284.

Parsons, R. J., C. E. Nelson, C. A. Carlson, C. C. Denman, A. J. Andersson, A. L. Kledzik, K. L. Vergin, S. P. McNally, A. H. Treusch, and S. J. Giovannoni. 2014. Marine bacterioplankton community turnover within seasonally hypoxic waters of a subtropical sound: Devil's Hole, Bermuda. Environmental Microbiology:DOI: 10.1111/1462-2920.12445.

Porter, K. G. and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr 25:943-948.

Santoro, A. E., K. L. Casciotti, and C. A. Francis. 2010. Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. Environmental Microbiology 12:1989-2006.

Data Processing Description

Analyses of the data were conducted using mothur version 1.33.3 (Schloss et al. 2009) and included contig construction of the paired ends, quality filtering, amplicon size selection (253bp median size) and alignment to the SSU rRNA gene. Chimera detection was conducted via UCHIME (Edgar et al. 2011) using mothur, and chimeric sequences were removed. Taxonomic classification of sequences was conducted in mothur with the SILVA SSU Ref database (version 117) using the k-nearest neighbor algorithm on sequences sub-sampled to the same depth with each primer pair (10,000, 12,000, or 17,500 sequences per sample for BIOS aquaria, Micronesia, and Red Sea sample sets, respectively). The sequencing error analysis, conducted on the mock community sequences amplified with the 515F/806RB primers, revealed a sequencing error rate of 0.0012%. Raw data are accessible in NCBI’s Short Read Archive under BioProject ID PRJNA279146.

References:

Edgar, R., B. Haas, J. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27:2194-2200.

Schloss, P. D., S. L. Westcott, T. Ryabin, J. R. Hall, M. Hartmann, E. B. Hollister, R. A. Lesniewski, B. B. Oakley, D. H. Parks, C. J. Robinson, J. W. Sahl, B. Stres, G. G. Thallinger, D. J. Van Horn, and C. F. Weber. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537-7541.

BCO-DMO Processing:

- 'not available' and 'not determined' changed to nd
- replaced 'n/a' with 'NA'
- replaced comma with semicolon
- corrected longitude for BIOS from 64 to -64

[table of contents | back to top]

Data Files

File
primers.csv (Comma Separated Values (.csv), 8.44 KB) MD5:37ebb936c8acf6a612f45b6abb20343c Primary data file for dataset ID 565723

[table of contents | back to top]

Parameters

Parameter	Description	Units
sample	sample name	unitless
depth	sample depth	meters
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
sample_descrip	description of location	unitless
pcent_SAR11	percent of SAR11 from FISH counts	percent
SAR11_stdev	standard deviation of SAR11 from FISH counts	percent
primr_806R	Linked sequence data file name of 515F/806R primers; at NCBI SRA	unitless
primr_806RB	Linked sequence data file name of 515F/806RB primers; at NCBI SRA	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	MiSeq (Illumina, San Diego, CA, USA)
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	AX70 epifluorescent microscope (Olympus, Tokyo, Japan)
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	
Generic Instrument Name	Fluorometer
Dataset-specific Description	Qubit 2.0 Fluorometer with the dsDNA High Sensitivity Assay (Life Technologies, Grand Island, NY USA)
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Bio-Rad thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA)
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)

[table of contents | back to top]

Deployments

Apprill_2013

Website	https://www.bco-dmo.org/deployment/565728
Platform	BIOS
Start Date	2012-09-15
End Date	2016-08-31
Description	High-throughput sequencing of small subunit ribosomal RNA (SSU rRNA) genes from marine environments. Coral-bacterioplankton mesocosm experiments.

[table of contents | back to top]

Project Information

Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

Coverage: Florida Keys, Federated States of Micronesia, Red Sea, & Bermuda

Description from NSF award abstract:
Reef-building corals are in decline worldwide due in part to climate change and other human activities, and it is becoming increasingly important to understand what aspects of coral biology are degraded by environmental stress which then leads to coral mortality. It is now widely known that corals harbor communities of bacteria and archaea that are believed to play important roles in maintaining the health of their hosts, but we lack any appreciable understanding about the identity of the microbial associates regularly residing within healthy, reef-building corals. This project asks the central question: do reef-building corals harbor fundamental or persistent microbial associates that are symbiotic within their tissues? In order to address this hypothesis, the investigator will assess the identity of the bacterial and archaeal microbes using a variety of molecular and microscopy approaches that includes the identification and localization of a widespread group of coral bacterial associates belonging to the genus Endozoicomonas. The results of this study will then be used to develop additional questions about the role of these microbial associates in nutrient cycling and how they contribute to the health and survival of corals.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233612

[table of contents | back to top]