V4 amplicon sequencing of coral tissue collected from Micronesia, the Florida Keys, and Australia in 2013 (Coral Microbial Relationships project)

Website: https://www.bco-dmo.org/dataset/662114
Data Type: experimental
Version: 1
Version Date: 2016-10-20

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

Contributors	Affiliation	Role
Apprill, Amy	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Gray, Laura	Woods Hole Oceanographic Institution (WHOI)	Contact
Ake, Hannah	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
V4 amplicon sequencing of coral tissue collected from Micronesia, the Florida Keys, and Australia in 2013 (Coral Microbial Relationships project)

Table of Contents

- Coverage
- Dataset Description
  - Methods & Sampling
  - Data Processing Description
- Data Files
- Parameters
- Instruments
- Deployments
- Project Information
- Funding

Coverage

Spatial Extent: **N**:24.606054 **E**:146.852 **S**:-15.0883 **W**:-81.39111

Dataset Description

Partial SSU rRNA genes of bacteria and archaea from tissue samples produced using 515F/806RB primers. Samples were collected in the Federated States of Micronesia, Austrailia, and the Florida Keys.

Methods & Sampling

Coral collection and processing

Coral fragments were collected by a scuba diver using a hammer and chisel during field sampling trips to Kapangamarangi Atoll, Micronesia (November 2012), Florida Keys, U.S.A. (May 2013) and Magnetic Bay, Australia (November 2013). Fragments were stored in a cooler containing ice until they were flash frozen in liquid nitrogen. Fragments were obtained from 3 representative colonies of the following species: *Porites lobata, Pocillopora verrucosa, Acropora humilis, Orbicella faveolata, Montastraea cavernosa,*

O. annularis, and Diploria strigosa. Fragments were shipped back to Woods Hole Oceanographic Institution and stored at -80 C until they were processed.

Using an airbrush, an aerosolized jet of sterile 1-X phosphate-buffered-saline (PBS) was directed at fragments. This method physically separated the coral mucus and tissue from the skeleton and suspended the cellular material in a slurry. The slurry was homogenized and centrifuged at 4 C for 20 minutes (5000 rpm) to form cellular tissue pellets. The PBS supernatant was removed and the tissue was evenly divided into smaller sections using sterile razors. To ensure that differing DNA yields were solely attributed to the lysis efficacy of the extraction treatments, the amount of biomass entering each extraction was standardized for all samples (38.69 +/- 9.23 mg). Subsampled biomass fractions were stored in separate tubes and frozen at -80 C until they were extracted.

Phase I DNA extractions

DNA was extracted from the subsampled biomass using the PowerSoil (cat # 12888), PowerPlant Pro (cat # 13400), PowerBiofilm (cat # 24000), and UltraClean Tissue & Cells (cat # 12334) DNA Isolation kits following the manufacturer's instructions (Mo Bio Laboratories, Inc.). In this study, the treatments are referred to by their abbreviations: PowerSoil (PS), PowerPlant® Pro (PP), PowerBiofilm® (PB), and UltraClean Tissue & Cells (UC). Manipulations to the mechanical lysis conditions for the UC extraction were made in phase II (see below), resulting in three permutations of the UC kit: Vortex Garnet (VG), Powerlyzer Glass (PG), and Vortex Glass (VGl). The optional proteinase-K digestion step (15 ul; 20 mg/mL at 60 C for 30 minutes) was implemented for all UC permutations. Samples were homogenized for 15 minutes using a vortex adaptor unless otherwise specified. Genomic DNA concentrations were assessed using the dsDNA High Sensitivity Qubit 2.0 flourometric assay (Life Technologies).

DNA template was screened for PCR efficiency using the barcoded primer pair 515F and 806RB[36]. Unaltered DNA template (0.18 – 47 ng l-1) was amplified in 25 l reactions containing 1.25 U of GoTaq Flexi DNA Polymerase (Promega), 5X Colorless GoTaq Flexi Buffer, 2.5 mM MgCl2, 200 M dNTP mix (Promega), and 200 nM of each barcoded primer in a Bio-Rad thermocycler (Bio-Rad Laboratories). The following PCR reaction conditions were used: 95 C for 2 minutes, followed by 40 cycles of 95 C for 20 sec, 55 C for 15 sec, and 72 C for 5 minutes, concluding with an extension step of 72 C for 10 minutes. PCR products were visually screened electrophoretically for quality using a 1% agarose/TBE gel under UV light. Positive PCR amplification was denoted by the presence of an amplicon band approximately 292 bp in size. Band size was assessed using the Hyperladder 50 bp DNA ladder (5 ng l-1) (Bioline).

Phase II: Mechanical lysis modifications

The phase I extraction treatment yielding extracts with the highest PCR efficiency for all coral colonies and species (UC) was selected to further examine if differences in bead type and homogenization method and duration resulted in intra-treatment extraction biases (Table 1). DNA was extracted from the same coral colonies used during phase I using the UC kit with minor modifications to the mechanical lysis conditions. The average biomass per extraction was 42.26 +/- 13.95 mg. For the first modification, the garnet beads provided with the kit were replaced with 0.1 mm glass beads (cat # 13118, Mo Bio Laboratories, Inc.) (VGl). For the second modification, a PowerLyzer 24 bench-top bead-based homogenizer (Mo Bio Laboratories, Inc., cat # 13155) was used to homogenize the tissue instead of the vortex adaptor and garnet beads were replaced with 0.1 mm glass beads (PG). Samples were homogenized with the Powerlyzer for 45 seconds at 3500 rpm. DNA was not extracted from three colonies (two *P. verrucosa* and one *A. humilis*) using the VGl treatment because of limited biomass. DNA concentrations were quantified and PCR efficiency assessed using the methods outlined above.

Library preparation and sequencing

Based on the phase I and II PCR screens, amplicons from the PB, VG, PG, and VGl extractions were selected for sequencing using the MiSeq platform (Illumina). In addition, two positive controls (*E. coli* DNA and the Human Microbiome Project mock community DNA (BEI Resources, cat # HM-276D)) and a negative control (U.V. sterilized DNA-free water) were amplified, barcoded, and included in the library pool. As an extra assessment of barcode reproducibility, each *O. annularis* extract was assigned two unique barcodes, amplified in separate reactions, and sequenced.

DNA template was amplified with the same V4 primer set [36], using similar PCR reaction conditions as those described above, but with 35 cycles. Amplicons were purified using selective gel purification (MinElute PCR Purification Kit, Qiagen) so that only PCR products matching the approximate size of the V4 SSU rRNA gene amplicon were included in the final library pool. Samples were prepared for sequencing using the methods outlined by Apprill and colleagues [1]. The amplicon pool was shipped to the University of Illinois W. M. Keck

Center for Comparative and Functional Genomics and sequenced using 2 x 250 bp paired-end MiSeq [1,2].

References

1. Apprill A, McNally S, Parsons R, Weber L. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat Microb Ecol. 2015;75(2):129-137; doi:10.3354/ame01753.

2. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol. 2013;79(17):5112-20; doi:10.1128/AEM.01043-13.

Sequence processing

Mothur software [1] (v.1.33.3) was used to combine the de-multiplexed paired reads (8,344,281 contigs) and remove longer sequences (>275 bp) and sequences containing ambiguous base pairs. A subset of sequences with read lengths exceeding 275bp were queried using the NCBI BLASTN 2.3.0 program [2,3] to evaluate top matches for these sequences. The remaining sequences were classified using the SILVA SSU Ref database [4] (v. 119) and sequences matching to Eukaryota, mitochondria, and ‘unknown’ lineages were discarded (2,802 sequences). Chloroplast sequences were retained to assess if more chloroplast sequences were associated with a particular DNA extraction treatment. The UCHIME algorithm [5] was used to identify and remove chimeric sequences (13,724 sequences total). Sequences were not subsampled [6,7].

The sequences were grouped using the Minimum Entropy Decomposition (MED) algorithm [8]. These MED nodes are analogous to operational taxonomic units (OTUs) and resolve biologically meaningful and distinct groups that can be separated by <1% sequence disparity [8]. Taxonomy was assigned to MED nodes using the classify.seqs command in mothur [1] and the SILVA database [4] (v. 119). Sequences belonging to ‘unclassified’ MED nodes were re-aligned using the SINA alignment service [9] (v. 1.2.11) and imported into ARB [10] (SSURefNR99_123). Using ARB [10], phylogenetic comparisons were made between the aligned sequences and sequences within the SILVA database [4] (v. 119) using neighbor joining algorithms in an attempt to resolve ‘unclassified’ taxonomy.

Data Processing Description

Statistical analysis

Data was tested for normality using the Shapiro-Wilk test. One-tailed t-tests were used for pair-wise comparisons after establishment of normality and equal variance. One-way ANOVAs were used for multiple group testing. If data failed normality, Friedman Repeated Measures Analysis of Variance (FRMANOVA) on ranks tests were conducted. When appropriate, Tukey’s, Holm-Sidak, or Dunn’s Method post-hoc tests were conducted to determine significantly different groups. P values ≤ 0.05 were accepted as being statistically significant. The above statistical tests were conducted using SigmaPlot software (v. 13).

Primer (v.7.0.9, Primer- E Ltd.) was used for prokaryotic community visualization and diversity analysis. Bray-Curtis distances were calculated from normalized, square root transformed sequence data and used to conduct non-metric multidimensional scaling (nMDS) and nested two- and one-way analysis of similarity (ANOSIM) tests. Presence/absence heat maps for O. faveolata, O. annularis, and A. humilis were created using the phyloseq [11] R package within the R-studio environment and a custom script [12] that was modified for this study. These heat maps were generated using distinct MED nodes that comprised 50% of all the reads obtained for each sample and thus represent the most dominant groups. Frequency of MED node detection was determined for each treatment and the percentage of detection agreement between pairwise treatments within each colony was assessed. One-tailed t-tests were used to reveal significantly different MED node detection between treatments.

References

1. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol. 2013;79(17):5112-20; doi:10.1128/AEM.01043-13.

2. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol. 2000;7(1-2):203-14; doi:10.1089/10665270050081478.

3. Morgulis A, Coulouris G, Raytselia Y, Madden TL, Agarwala R, Schäffer AA. Database indexing for production MegaBLAST searches. Bioinformatics. 2008;24:1757-64; doi: 10.1093/bioinformatics/btn32.

4. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(D1):D590-D596; doi:10.1093/nar/gks1219.

5. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-200; doi: 10.1093/bioinformatics/btr381.

6. de Ca´rcer DA, Denman SE, McSweeney C, Morrison M. Evaluation of subsampling-based normalization strategies for tagged high-throughput sequencing data sets from gut microbiomes. Appl Environ Microbiol. 2011;77(24):8795–8798; doi: 10.1128/AEM.05491-11.

7. Holmes S, McMurdie PJ. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput Biol. 2014;10(4):e1003531; doi:10.1371/journal.pcbi.1003531.

8. Eren AM, Maignien L, Sul WJ, Murphy LG, Grim SL, Morrison HG, Sogin ML. Oligotyping: differentiating between closely related microbial taxa using 16S rRNA gene data. Methods Ecol Evol. 2013;4:1111–1119; doi:10.1111/2041-210X.12114.

9. Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics. 2012;28:1823-1829; doi: 10.1093/bioinformatics/bts252.

10. Ludwig, W, Strunk O, Westram R, Richter L, Meier HY, Buchner A, Lai T, Steppi S, Jobb G, Förster W, Brettske I, Gerber S, Ginhart AW, Gross O, Grumann S, Hermann S, Jost R, König A, Liss T, Lüßmann R, May M, Nonhoff B, Reichel B, Strehlow R, Stamatakis A, Stuckmann N, Vilbig A, Lenke M, Ludwig T, Bode A, Schleifer KH. ARB: a software environment for sequence data. Nucleic Acids Res. 2004;32(4):1363-1371; doi:10.1093/nar/gkh293.

11. McMurdie PJ, Holmes S. Phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. PLoS ONE. 2013;8(4):e61217; doi:10.1371/journal.pone.0061217.

12. Neave MJ. MEDecomposition. GitHub repository. https://github.com/neavemj/globalCoralMicrobiomes. Accessed 15 November 2015.

BCO-DMO Data Processing Notes:

-removed spaces and replaced with underscores
-reformatted column names to comply with BCO-DMO standards
-converted all lat/lons to decimal degrees
-added accession links
-filled in blank cells with nd

[table of contents | back to top]

Data Files

File
coral_accessions.csv (Comma Separated Values (.csv), 11.72 KB) MD5:9cb173adf7e91b98688fa97944d5384e Primary data file for dataset ID 662114

[table of contents | back to top]

Parameters

Parameter	Description	Units
coral_colony	PI issued ID for the coral colony sampled	unitless

coral_species	Species of coral sampled	unitless
location	Geographic location where sampling occurred	unitless
depth	Depth where sampling occurred	meters
lat	Latitude; N is positive	decimal degrees
lon	Longitude; W is positive	decimal degrees
UCT_CP_glass_accession	Accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition	unitless
UCT_CP_glass_accessionLink	Accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition	unitless
UCT_CP_glass_replicate_accession	Replicate accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition	unitless
UCT_CP_glass_replicate_accessionLink	Replicate accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition.	unitless
UCT_CV_garnet_accession	Accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex garnet (VG) beads permutation.	unitless
UCT_CV_garnet_accessionLink	Accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex garnet (VG) beads permutation.	unitless
UCT_CV_garnet_replicate_accession	Replicate accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex garnet (VG) beads permutation.	unitless
UCT_CV_garnet_replicate_accessionLink	Replication accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex garnet (VG) beads permutation.	unitless
PB_V_accession	Accession number for DNA extracted from subsampled biomass using PowerBiofilm (PB) Isolation Kit	unitless
PB_V_accessionLink	Accession link for DNA extracted from subsampled biomass using PowerBiofilm (PB) Isolation Kit	unitless
PB_V_replicate_accession	Replicate accession number for DNA extracted from subsampled biomass using PowerBiofilm (PB) Isolation Kit	unitless
PB_V_replicate_accessionLink	Replicate accession link for DNA extracted from subsampled biomass using PowerBiofilm (PB) Isolation Kit	unitless
UCT_CV_glass_accession	Accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex glass (VGI) beads permutation.	unitless
UCT_CV_glass_accessionLink	Accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex glass (VGI) beads permutation.	unitless

UCT_CV_glass_replicate_accession	Replicate accession number for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex glass (VGI) beads permutation.	unitless
UCT_CV_glass_replicate_accessionLink	Replicate accession link for DNA extracted from subsampled biomass using Ultra Clean tissue and cells (UC) Isolation Kit; manipulation to the mechanical lysis condition Vortex glass (VGI) beads permutation.	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Airbrush with 80 psi air pressure
Generic Instrument Name	Airbrush
Dataset-specific Description	Frozen coral tissue was removed from skeleton using airbrush.
Generic Instrument Description	Device for spraying liquid by means of compressed air.

Dataset-specific Instrument Name	PowerLyzer 24 Bench-Top Bead-Based Homogenizer
Generic Instrument Name	Homogenizer
Dataset-specific Description	Used to homogenize the coral tissue
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Bio-Rad thermocycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Reactions carried out on a 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]