Elucidating the black coral microbiome using amplicon sequencing

UNIVERSITY OF SOUTH CAROLINA BEAUFORT

Yessenia Bledsoe-Becerra[1], Jicayla Johnson-Rosemond[1], Iesha Whittaker[1], Kristen Mullins[1], Karena Cunningham[1], Abigail Ahlers[1], Mercer R. Brugler[1^]

[1] Department of Natural Sciences, University of South Carolina Beaufort, 801 Carteret Street, Beaufort, SC 29902 USA

Introduction

The microbiome is the sum of microscopic organisms inhabiting a host. These organisms include bacteria, protists, viruses, fungi, etc., and can be found within and on an organism. Learning about the microbiome of black corals will help researchers understand important ecological factors such as how microbes promote survival through means of nutrient production, mineral fixing/recycling, immune protection, and many other important functions. According to the holobiont theory, microbiomes even affect the evolution of a host. With the decline of corals world-wide, a special interest in how microbial communities influence the overall health of corals has been established. In this study, we are utilizing amplicon sequencing to characterize the microbiome of black corals collected from the Flower Garden Banks National Marine Sanctuary in the northwest Gulf of Mexico to expand our ecological and evolutionary knowledge of black corals.

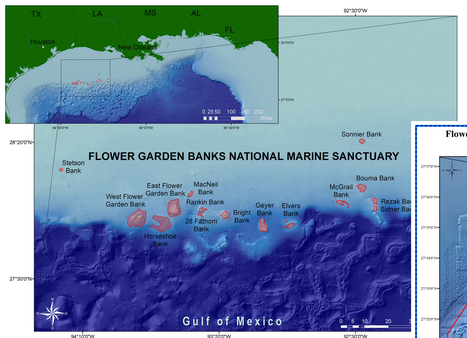

Figure 1. A map of the Flower Garden Bank area NOAA (2021).

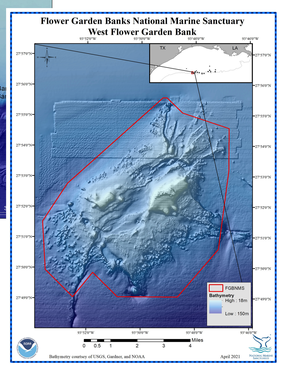

Figure 2. Expanded view of the West Flower Garden Bank NOAA (2021).

Figure 3. Students photographed with rover Yogi, used to collect samples.

Objectives

Target microbes using genes:

- 16S (V4) to amplify bacteria and archaea
- ITS1 to amplify fungi
- 28S (D2/D3) to amplify protists
- 18S to amplify microscopic metazoans
- polB (DNA polymerase) to amplify viruses

Figure 4. *Tanacetipathes sp., a* representative in this study Wicksten et al. (2014).

Materials and Methods

- Specimens: *Elatopathes abietina, Acanthopathes thyoides, Antipathes furcate, Tanacetipathes sp., Antipathes atlantica, Stylopathes cf. litocrada, Acanthopathes sp.*
- DNA Extraction: Puregene® Core Kit A
- PCR: TaKaRa Ex Taq®
- DNA quantification: Quibit™ dsDNA HS Assay kit, Qubit 4.0 Fluorometer
- Amplicon-EZ sequencing through Genewiz® (50,000 sequences per gene region)

Figure 5. *Acanthopathes thyoides,* a representative in this study Opresko (2016).

Results

- Initial PCR gel
  - Blank due to co-extracted inhibitors
- Troubleshooting
  - Dilute DNA to combat co-extracted inhibitors
  - Tested dilutions 1:5, 1:10, 1:20, 1:30, 1:50, 1:100, 1:150
    - 1:20 provided the clearest band
- At Present
  - Optimizing annealing temperatures to decrease non-specific binding of primers

Conclusions

Subsequent to troubleshooting and optimizing the experiment, PRC products will be sequenced by Genewiz®.

Our DNA sequence data will be compared to that of Liu et al. (2018) who recovered 52 bacterial and 3 archaeal phyla in black corals *Antipathes ceylonensis and A. dichotoma.*

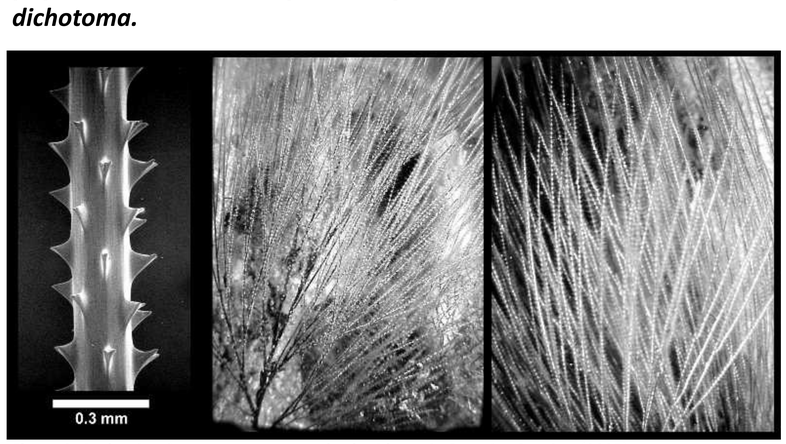

Figure 6: *Antipathes furcate, a* representative in this study Opresko, Sanchez (2005).

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