The Biodiversity of Green Fluorescent Coral Zooxanthellae

By Nathalie Alvarez, Mason Peretson, and Frederick Feraco

Walt Whitman High School

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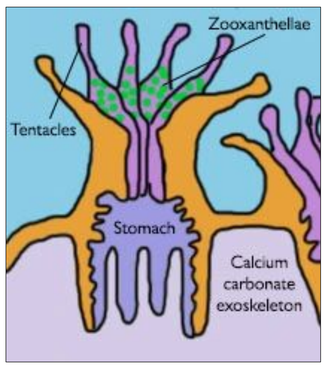
Abstract

This project is a continuation of our previous works, we identified the fluorescence of a multitude of coral. We took inspiration from our past mentor, Charles Mazel, who focused on the potential medical applications of zooxanthellae protein, which we attempted to prove at first. Unfortunately we ran into some errors last year which prompted us to change a few things. We followed the Cold Spring Harbor DNA extraction protocol to extract and purify DNA from coral tissue. Subsequently, we amplified a specific region of the chloroplast using polymerase chain reaction (PCR). However, the sequencing did not yield the expected results. We believe this failure was due to our storage method rather than the extraction process. Next year, we plan to adopt a more effective storage method to improve our outcomes.

Introduction

- Our project is to identify the different species of zooxanthellae produced by corals containing green fluorescent protein.
- We wanted to determine if every green fluorescent coral contains the same species. We were able to identify GFP (green fluorescent protein) in a few of the corals in our school's coral tank, mainly Green Acropora and other SPS corals.
- Our current objective is to DNA barcode the zooxanthellae and to successfully culture the proteins that would be extracted.
- Our hypothesis is completely based on the successful extraction of protein from zooxanthellae.
- We were inspired by our previous project and information from our mentor, Charles Mazel.



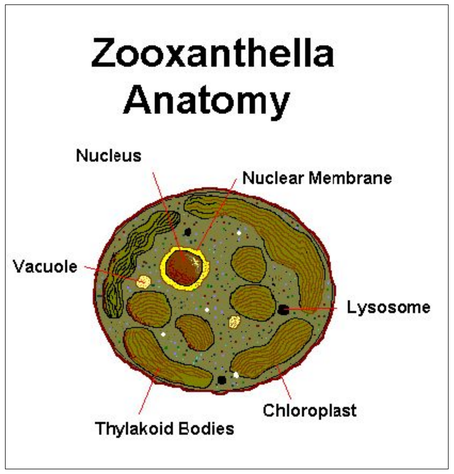
Materials & Methods

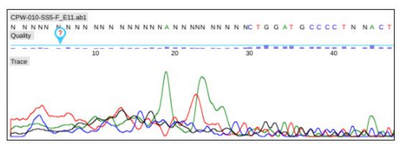
We have access to a variety of corals through our school's coral tank, which many contain the green fluorescent proteins that were produced by their zooxanthellae.

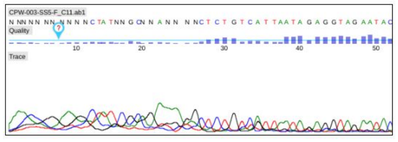
1. We would select a fragment of the coral who's zooxanthellae we would want to identify and observe.
2. Then we would attempt to isolate the zooxanthellae through the use of a centrifuge, which divides substances based on density.
3. We would then put the now centrifuged zooxanthellae under a microscope where we are able to photograph it.
4. We then went through the Cold Spring Harbor DNA extraction procedure extracting and purifying the DNA from the coral tissue followed by amplifying a specific region of the chloroplast by adding polymerase chain reaction (PCR).
5. We then performed gel electrophoresis on the samples collected while simultaneously combining them with different primers. We used three different primers including COI invertebrate specific primer, tufA primer, and a ss5z + ss3z mixture.

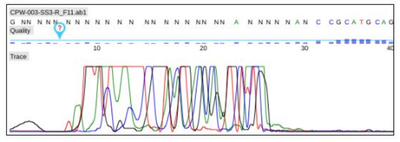
Results

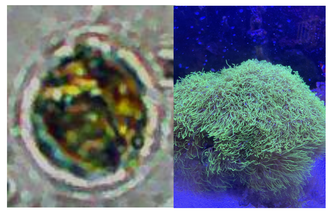
1. *1st graph has a lot of missing base pairs, possibly because it was one of the older samples and was stored in the freezer for too long*
2. *2nd graph also has a lot of missing pairs, possibly because it was one of the older samples and was stored in the freezer for too long*
3. *3rd graph has the most similar base pairs, however couldn't be fully sequenced which may be due to the time in the freezer as well.*



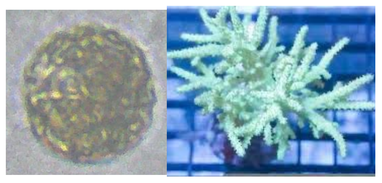




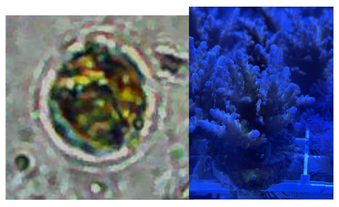




Green Star-Polyp Zooxanthellae



Green Finger-leather Zooxanthellae



Green Acropora Zooxanthellae

Discussion

- We studied the comparison of zooxanthellae in similar fluorescing corals
- This study was expected to be important to determine if the zooxanthellae could be farmed using similarly colored corals, and eventually we would look for alternative uses for the zooxanthellae
- Our hypothesis wasn't right nor wrong, due to the insufficient results we derived
- We determined that our extraction methods were capable of extracting the zooxanthellae successfully, however our storage methods were flawed
  - This is because our imaging displayed successful zooxanthellae extraction, but the sequencing displayed insufficient results, which were stored in saltwater, determining that saltwater was the improper liquid to store it in
- We can attempt to use a different method of storage for better specimens to sequence and/or send the samples in for sequencing sooner.
- Next year, we will enhance our research in the outside uses for zooxanthellae in the biology and medical field along with increasing our sample sizes
- We learned that our extraction methods were successful, but our storage methods weren't

References

Valdivia, R. H., Hromockyj, A. E., Monack, D., Ramakrishnan, L., & Falkow, S. (1996). Applications for green fluorescent protein (GFP) in the study of hostpathogen interactions. Gene, 173(1), 47–52. https://doi.org/10.1016/0378-1119(95)00706-7

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Acknowledgements

Thank you for all the support provided by Mr. Feraco over the past four years.

Abstract

This is project is a continuation of our previous project in which we identified the fluorescence of many corals. Previously, one of our mentors, Charles Mazel, told us that the proteins had the possibility of being used for medicine, which is what inspired our current project in the first place. We went through the Cold Spring Harbor DNA extraction procedure extracting and purifying the DNA from the coral tissue followed by amplifying a specific region of the chloroplast by adding polymerase chain reaction (PCR). The sequencing, however, wasn't as successful as we hoped. We believe the reason for its failure was due to the method of storage instead of our method of extraction. We will attempt a more effective way of storage for the following year.

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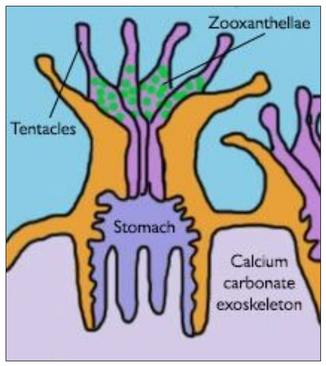

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