Using DNA Barcoding to Decipher the Marine Invertebrates that are Living on Macroalgae

Authors: Rachel Denzler[1], Catherine Fregosi[1], Sara O'Sullivan-Bakshi[1]
Mentors: Mary Kroll[1], Cristina Fernandez-Marco[2], Sharon Pepenella[2]
[1]West Islip High School; [2]Cold Spring Harbor Laboratory's DNA Learning Center

Cold Spring Harbor Laboratory DNA Learning Center
National Institutes of Health SEPA Science Education Partnership Award
Supported by the National Institutes of Health

Abstract

Algae plays an important role in the ecosystems of waterways in our community. Determining relationships between algae and marine invertebrates living in symbiosis is critical to our understanding of biodiversity. Samples were collected from Gardiner Park. DNA was extracted from each sample, and amplified by PCR using the rbcl gene for algae and the CO1 gene for invertebrates. Electrophoresis was performed and eleven positive results were sent to Cold Spring Harbor Laboratory for sequencing. DNA Subway was used to interpret the sequencing results. Two samples were found to be unknown. Six samples were found to be either *Silurus astous*, *C. deodara*, *Ulva flexuosa*, or *Zostera marina*. All of these algae provide habitats for invertebrates to thrive. Future research would include re-experimentation to gather more DNA results from the invertebrates. Ideally, understanding what each organism needs from the algae would be gained, giving clarity to the Great South Bay ecosystems. Once a more clear understanding of the beginning of our food chains is achieved, making it easier to conclude what changes should be made to ensure human health.

Introduction

- The objective of this project was to draw conclusions about the types of symbiotic relationships between marine invertebrates and the algae collected, ultimately allowing further investigation of the Great South Bay's ecosystems.
- The Great South Bay is along the south side of Long Island N.Y., is about 50 km long, 10 km wide, and has an area of about 50,000 acres, which limits the rise and fall of tide in the bay to about 60 cm (Mackenzie, 2003). There has also been an increase in non-native algae growth in environments off the coast of Long Island, leading to the effect of a decrease of microphyte biomass as compared to its native habitat (Janiak & Witlatch, 2012). Microphyte biomass is the total mass of microscopic algae, typically found in freshwater and marine systems living in both the water column and sediment.
- The goal of this investigation was to learn of the different species of marine invertebrates living on the algae that were collected from the Great South Bay and how their presence is relevant to the stability of their ecosystem.
- Based on the initial observations and readings from articles, it was hypothesized that the organisms found living on the algae will have a mutualistic relationship, having a neutral effect on the environment.

Materials and Methods

Macroalgae samples were collected from Gardiner Park from 4 posts. They were then photographed and documented at the lab.

DNA extraction was performed by the silica method.

The PCR protocol was followed using CO1 for invertebrates RBCL for algae.

Gel electrophoresis was performed, and positive results were sent to Cold Spring Harbor Laboratory for sequencing.

DNA Subway was used to interpret the sequenced results.

Results

- The results of this research project showed no DNA was extracted from samples the first time barcoding.
- The DNA was run through gel electrophoresis a second time and no results were obtained.
- The DNA extracted from algae and invertebrates were sent to CSHL for evaporation to concentrate the DNA, then re-amplified at Cold Spring Harbor Laboratory.
- Invertebrate samples 14 and 15 showed results after Cold Spring Harbor Lab's DNA Learning Center concentrated the samples using a rotary evaporator.
- The same was done to algae samples 1, 7, 17, 18, 19, and 20.
- After the second trial for the DNA Barcoding, we obtained 8 positive results.
- The samples were sequenced and then DNA Subway was used to interpret the sequences.
- 2 samples were found to be unknown.
- 6 samples matched the sequencings of *Silurus astous*, *C. deodara*, and *Ulva flexuosa* to samples in the GenBank.

Table: Metadata and DNA Subway results for sequenced samples

Sample ID	Common Name	Habitat Description	Salinity of Water (PPT)	Nitrate Levels (PPM)	Bit Score	E Value	Mis-matches	Scientific Name
PHF-001	Algae	Murky water, shallow bank near dock, wood	25.4	0.0-2.5	432	9e-118	43	Unknown-high e-value, may not be good sequence
PHF-007	Algae	Muddy, shallow, thin shoreline, wood	29	0.0-1.5	843	0	1	Ulva flexuosa
PHF-014	Inverts	Sandy, muddy bank, wood	27	1	906	0	19	Silurus ssotus
PHF-015	Inverts	Sandy, muddy bank, wood	27	1	791	0	33	high mismatches due to low quality sample
PHF-016	Inverts	Muddy, sandy, dirt shoreline, wood	26.3	2	695	0	38	high mismatches due to low quality sample
PHF-017	Algae	Muddy, sandy, dirt shoreline, wood	26.3	2	834	0	0	Zostera marina
PHF-018	Algae	Muddy, sandy, dirt shoreline, wood	26.3	2	726	0	1	Ulva flexuosa
PHF-019	Algae	Muddy, sandy, dirt shoreline, wood	26.3	2	726	0	11	C. deodara RBCL
PHF-020	Algae	Muddy, sandy, dirt shoreline, wood	26.3	2	868	0	4	Ulva flexuosa

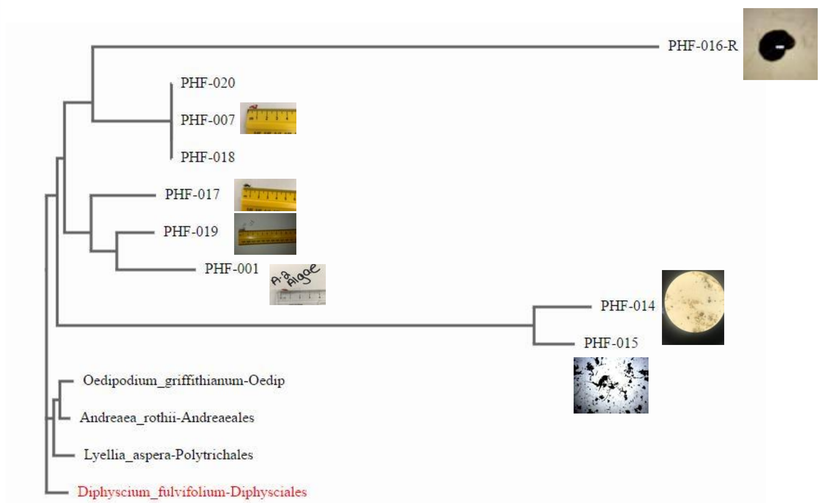

Figure: Phylogenetic tree of sequenced and reference samples

Discussion

- This experiment gave an understanding of the biodiverse ecosystems on the shores of Long Island. The mutualistic relationship found between algae and micro invertebrates furthered knowledge about our local ecosystems, which ultimately leads to affecting human health.
- 3 samples were unknown, although the sequences were low quality so the high number of mismatches does not mean a novel barcode was found.
- 6 samples matched samples in GenBank. The 6 samples were found to be Silurus astous, C. deodara RBCL, Ulva flexuosa or Zostera marina
- Ulva flexuosa is an algae species found worldwide, typically transported and spread by ships (M.D. Guiry, 2017).
- Zostera marina, an algae native to North America which people have long used as roof thatching, fertilizer, and also dried and used as stuffing for mattresses and furniture (Short, 2017).
- One sample was found to be most closely matched with C. deodara RBCL, which is a himalayan cedar (Aljos, 1990).
- The sample found to be Silurus astous is known as the Amur catfish (Sugita, 2007). This species in not native to the Great South Bay either. Researchers hope to determine why and how this was found in the GSB in the future.
- The three unknown species are unknown because the amount of mismatched nucleotides in the sequencing. These sequences were also low quality which is why there were a lot of mismatches.
- In the future we hope to re-do the extraction to determine what samples they are, and hopefully barcode more invertebrate samples. This will help to gain a further understanding of the relationships between the invertebrates and algae.

References

Aljos, Farjon (1990). *Pinaceae: drawings and descriptions of the genera Abies, Cedrus, Pseudolarix, Keteleeria, Nothotsuga, Tsuga, Cathaya, Pseudotsuga, Larix and Picea*. Koenigstein: Koeltz Scientific Books. ISBN 978-3-87429-298-6

Janiak, D.S., & Witlatch, R. B. (2012). Epifaunal and algal assemblages associated with the native Chondrus crispus (Stackhouse) and the non-native Grateloupia turuturu (Yamada) in eastern Long Island Sound. *Journal of Experimental Marine Biology and Ecology*, 413, 38-44.

Mackenzie, C. L., Jr. 2003. Comparison of invertebrate abundances in four bays of the northeastern United States: two bays with sparse quahogs and two bays with abundant quahogs. Northeast Fish. Sci. Cent. Ref. Doc. 03-10; 25 p.

M.D. Guiry in Guiry, M.D. & Guiry, G.M. 2017. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 23 May 2017.

Short, F.T., Carruthers, T.J.R., Waycott, M., Kendrick, G.A., Fourqurean, J.W., Callabine, A., Kenworthy, W.J. & Dennison, W.C. 2010. *Zostera marina*. The IUCN Red List of Threatened Species 2010: e.T153538A4516675. http://dx.doi.org/10.2305/IUCN.UK.2010 3.RLTS.T153538A4516675.en. Downloaded on 23 May 2017.

Sugita, H., Ohta, K., Kuruma, A., & Sagesaka, T. (2007, June 06). *An antibacterial effect of Lactococcus lactis isolated from the intestinal tract of the Amur catfish, Silurus asotus Linnaeus*. Retrieved May 24, 2017, from http://onlinelibrary.wiley.com/wol1/doi/10.1111/j.1365 2109.2007.01765.x/full

Acknowledgements

We would like to thank our teacher Mrs. Kroll and the teachers that helped us during our field trips who were, Ms. Munno, Mr. Rapczyk and Mrs. Nohowec. Also we would like to thank Cold Spring Harbor Laboratory for helping us through our experimentation.