Distinct Bacterial Populations Colonizing Plastic Debris in Coastal Waters of Southern California

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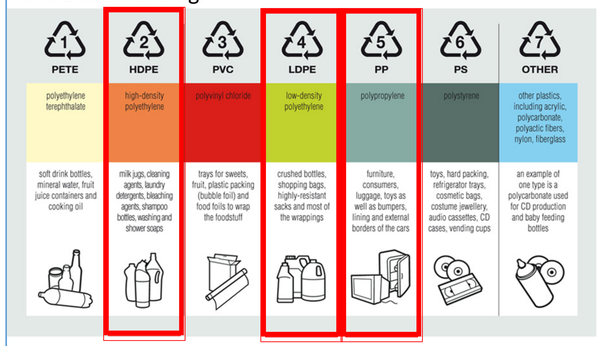
Introduction

The plastisphere is a unique ecosystem of microbes that attach to plastic in the ocean. It has been proven that these microbes impact the the ocean and its inhabitants. The damaging effects of plastic on the marine environment have been comprehensively studied. While the microbial populations present on microplastics, tiny fragments resulting of physical and chemical breakdown of plastic debris, have been described in multiple studies, much less is known about the initial stages of bacterial colonization. Our project investigates the microbes growing on plastic deployed in two different location (Dana Point/ Doheny State Beach and La Jolla/ Scripps Pier) for periods ranging from 0 to 40 days.

Objective

The objective of this research is to identify and functionally characterize microbes collected from plastic incubated in ocean water using live culture experiments and 16S barcoding.



Materials and Methods

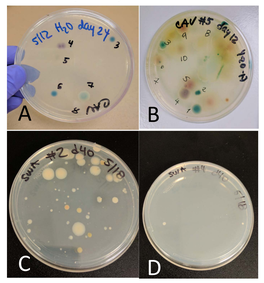
Three different plastics (HDPE, LDPE, and PP) were sterilized with ethanol and treated with UV light and all metal components of project were autoclaved. Plastic samples were zip tied, placed within a metal cage, and deployed into the ocean. Deployment samples are collected, and a water sample is collected for testing.

To the left is a cage ready to be deployed in the the ocean water.

To the right is a cage that has been recovered from the ocean water.

Cultures

Once plastic is collected from cages in the ocean, cultures are started on Saltwater Agar (SWA) and Chromagar Vibrio (CAV) Media.



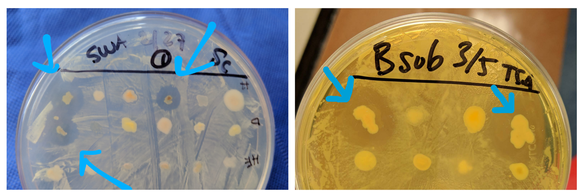
A. Water bacterial colonization on CAV
B. Polypropylene bacterial colonization on CAV
C. Low-density Polyethylene on SWA
D. High-density Polyethylene bacterial colonization on SWA

DNA and PCR Analysis

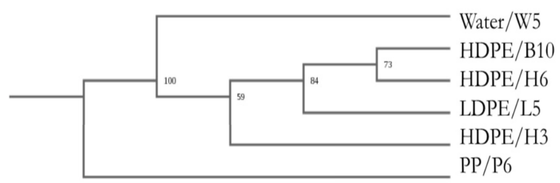
DNA was isolated from colonies using the Biostic kit (Qiagen) Amplification was completed using 16S primers 27F and 1492R.Sanger Sequencing performed by Genewiz allowed for sequence analyzation to be completed using DNA Subway.

Antibiotic Production

Colonies were patched onto bacterial lawns of both Gram-positive and Gram-negative organisms. The presence of a zone of inhibition was considered a positive result.

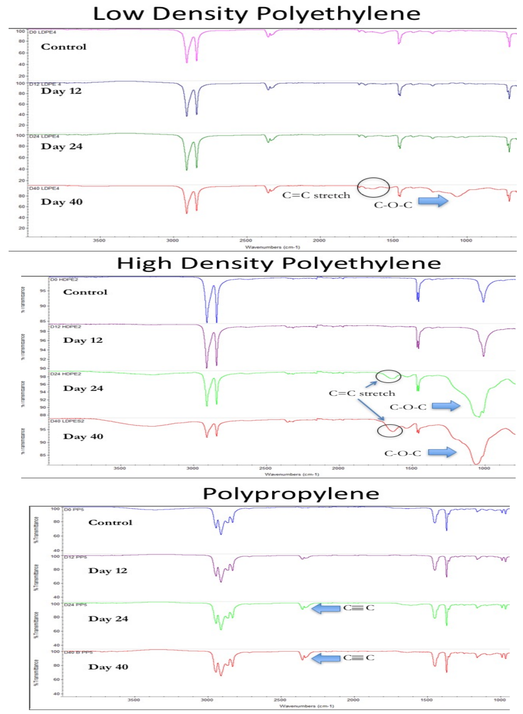


So far, 6 distinct bacteria have been isolated from both water and plastic samples. All products are alpha-proteobacterial, mostly in the genus *Phaeobacter.*



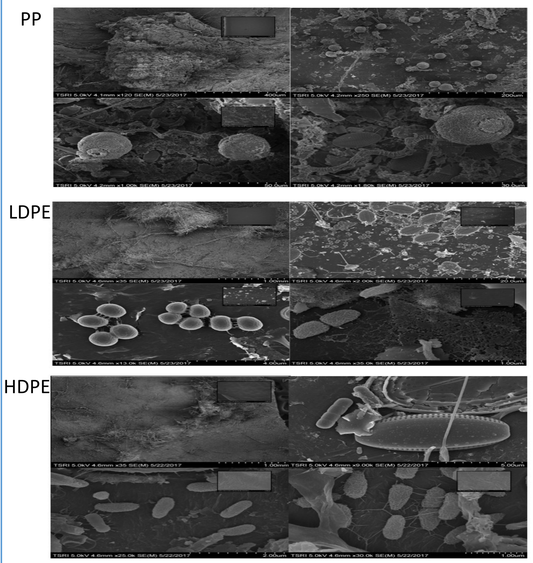
Fourier Transform Infrared Spectroscopy

FTIR was used to analyze the breakdown of plastic over time. Signs of oxidation of the plastic are apparent, especially in the HDPE sample.

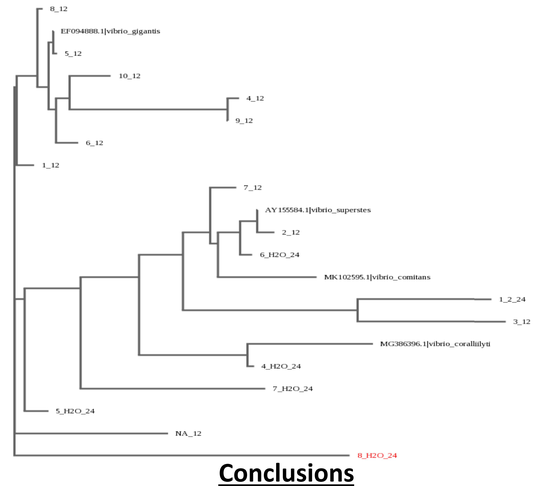


Scanning Electron Microscopy

Scanning electron microscopy was completed in order to visualize the biofilms attached to plastic samples that were in the ocean for 40 days.



Phylogenetic Tree



Conclusions

- This study indicates that there are differences in the microbial communities growing on coastal marine plastic debris with potential implications for human health and the environment.
- After initial sequencing with only 27F primer, the samples were rerun to include both 27F and 1492R primers. This showed a more complete sequence.
- Chemical analysis needs to be continued on the antibiotic producing microbes. Further testing can reveal the functional characteristics.
- Whole genome sequencing of the antibiotic producers needs to be completed for data mining and identification.

References

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doi:10.1021/es401288x

Acknowledgements

The authors would like to thank Dr. Jeff Bowman and his lab at SIO, Dr. Emelia DeForce, and NU students that are vital to the success of this project.

This project has been supported by NU internal grants as well as the NSF HIS's new to NSF award #1832545Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation