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Comparison of Bacterial Communities on Different Substrate Types in an Intertidal Anthropogenic Sulfide Seep

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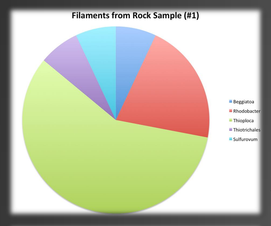
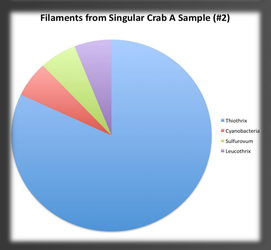
Abstract:

Mats of sulfide-oxidizing bacteria can typically be found surrounding high sulfide hydrothermal vents created by the separation of tectonic plates on the ocean floor. These bacteria have been known to form symbiotic associations with crustaceans such as the recently discovered Yeti Crab. An analogous high sulfide environment has been discovered in Commencement Bay in the form of an anthropogenic sulfide seep. Several species of crabs hosting bacterial communities on their carapace thrive in this high sulfide environment. 16s rRNA analysis and fluorescence in situ hybridization suggest that the phylogenies of the bacterial communities on the rocks and on the crabs in Commencement Bay are composed of significantly different bacterial communities. These results were confirmed by light microscopy and size diversity study. Sediment samples were also sequenced, revealing a highly diverse community of sediment bacteria surrounding the rocks and crabs, which also differs significantly in the phylogenies of its bacterial residents. This study has also revealed the presence of a as-yet unidentified small filamentous bacterium which resides on both the rocks and the crabs

Background:





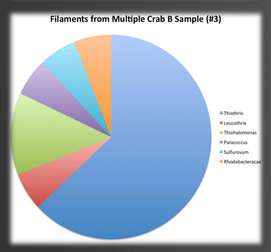


Figure 1. Results of Sequencing for crabs and Rocks

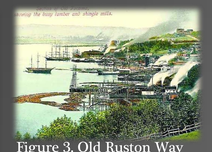


Figure 3. Old Ruston Way

Figure 4. A Yeti Crab

Between 1869 and 1977, Tacoma's Ruston Way filled with factories, smelters, and wood mills (Nerheim 2004) (figure 3). These wood mills produced large amounts of sawdust, which was economically disposed of by burying it in Commencement Bay. Currently, this sawdust is undergoing decomposition by sulfate-reducing bacteria, which produce large amounts of sulfide—up to 3944 µm (Elliott et al. 2006); a concentration 35 times higher than the 110 µm found at hydrothermal vents near the Galapagos islands (Grieschaber et al. 1998).

Katie Barton studied this area and discovered:

•Crabs can be found in these sulfide streams that have bacterial filaments growing on their carapace.

•the bacterial community on the rocks in the sulfide seep is comprised mostly of White Point (figure 1).

•The bacteria growing on crabs living in the sulfide seep is comprised mostly of *Thiothrix* (figure 1).

• *Thiothrix* is known to form symbiotic relationships with other organisms, in including the "Yeti crabs" found at hydrothermal vents(Rieley et. al., 1999)

Figure 2. Bacterial Filaments on rock and crab substrate

Research Goals:

1. Document and compare the microbial diversity of bacterial communities on the rocks and crab carapace.
2. Evaluate if differences between the microbiota found on the crab carapace and rocks is due to substrate preference.
3. Examine possible bacterial substrate preference for rocks or crab carapace.

Methods and Materials:

Phylogenetic Analysis:

•Sediment samples for sequencing were previously prepared through DNA extraction by Katie Barton

•Sediment DNA was amplified by PCR.

•A TA cloning reaction was then performed using TOP 10 pre-competent cells.

•34 successful colonies were selected, and minipreps were performed to extract DNA and insert the desired DNA into a plasmid.

Methods and Materials Continued:

fluorescence In Situ Hybridization

•Samples were prepared by fixing in 4% paraformaldehyde solution and were scraped and washed with distilled water on to nitrocellulose paper.

•FISH probes for *Thiothrix* and White Point were hybridized onto Crab and Rock filament samples on nitrocellulose paper. The samples were counterstained with DAPI and the number of *Thiothrix* or White Point filaments present was compared to the number of DAPI stained filaments present, and the ration of *Thiothrix* or White Point to overall bacterial filaments was established.

Size Diversity Study

•The width and length of the cells of 100 filaments from two different rock samples and one crab sample were measured under light microscopy.

Results:

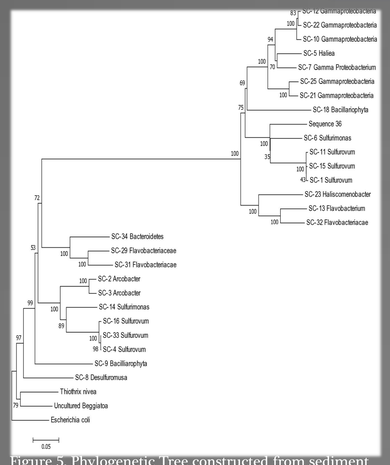


Figure 5. Phylogenetic Tree constructed from sediment sequencing data

Results of sequencing:

•Sediment samples are extremely diverse and are distributed into two distinct clades.
Sediment samples do not show evidence of *Thiothrix* or White Point; these bacteria are specific for crab carapace and rock substrate (fugure 5, figure 6).

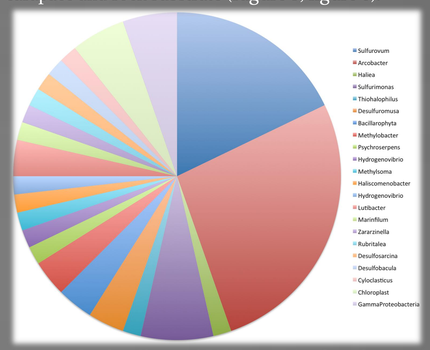


Figure 6. Results of bacterial DNA sequencing from the sediment

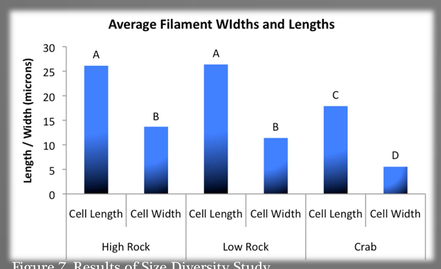


Figure 7. Results of Size Diversity Study

Size Diversity Study:

•Crab filaments were found to be significantly smaller than both rock filament samples found high on the tide and rock filaments found low on the tide (ANOVA, $P < 0.001$) (figure 7).

•Rock filaments high on the tide and low on the tide were not found to be significantly different (ANOVA, $P = 0.05$) (figure 7).

Results of fluorescence In Situ Hybridization:

- 40.1% of the filaments on the crab carapace were found to be *Thiothrix*.
- 4.68% of the filaments on the crab carapace were found to be White Point
- 38.6% of the filaments on the rocks were found to be White Point.
- 10.4% of the filaments on the rocks were found to be *Thiothrix*.
- Sequenced samples were plucked from rocks and crabs. FISH samples were scraped onto nitrocellulose paper.
- Further analysis did not reveal a significant difference between plucked and scraped samples.

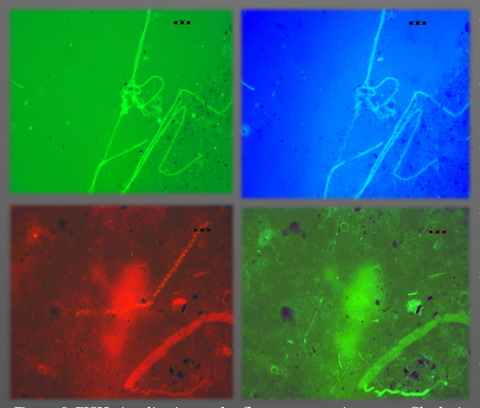


Figure 8. FISH visualization under fluorescence microscopy. Clockwise from the upper left: GFP showing *Thiothrix*, DAPI showing all bacterial filaments, FITSI showing White Point , GFP showing *Thiothrix*.

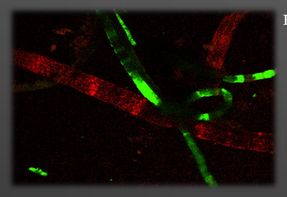


Figure 9. FISH visualization using confocal microscopy. Green represents *Thiothrix*, red represents White Point.

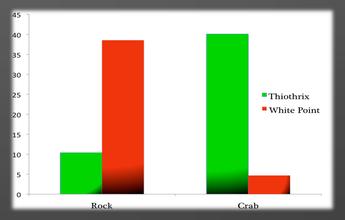


Figure 10. Summary of FISH analysis

Conclusions:

•Dominance of *Thiothrix* and White Point in the bacterial communities on the rocks and crabs are not as complete as previously thought.

•According to sequencing analysis, *Thiothrix* should make up 60% - 80% of the bacteria on the crabs and White Point should make up about 60% of the bacteria on the rocks.

•FISH analysis reveals that about 40% of the bacteria on the rocks are *Thiothrix* and about 40% of the bacteria on the crabs are White Point.

•Size diversity studies confirm that the size of bacteria on the crabs is significantly smaller than the size of the bacteria on the rocks, indicating a higher density of the smaller *Thiothrix* on the crabs and a higher density of large White Point filaments on the rocks.

•An entirely different community of bacteria were found on glass slides planted in the sulfide seep, showing conclusively that bacterial density is substrate dependent.

•Sediment bacterial communities are highly diverse and include sulfur oxidizers as well as cyanobacteria and diatoms.

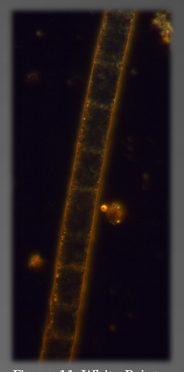


Figure 11. White Point

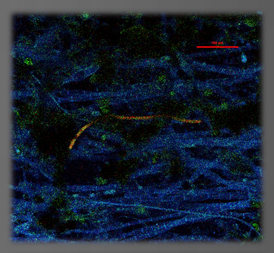
Future Study:

This study has indicated the presence of an unidentified large bacterium, about 10 microns in diameter (figure 12, 13). This bacterium contains sulfur granules, and grows in white, attached filaments. It has been seen growing on rocks and on glass slides placed in the sulfide seep.

In order to determine the identity of this bacterium, we will use another FISH probe to further narrow down the possibilities and we will attempt to isolate the filaments of this bacterium and sequence them.

Further studies will also investigate the reasons why these bacteria exhibit substrate dependence; is the reason metabolic? Do certain bacteria attach better to some substrates than to others?

Also, further studies will attempt to conclude, definitively whether or not there is a symbiotic relationship between *Thiothrix* and shore crabs.



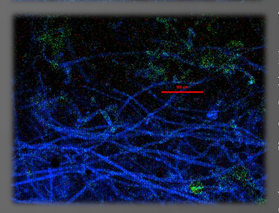


Figure 12. Confocal Microscopy of slide left in sulfide seep, revealing a large number of filaments not stained with White Point or *Thiothrix* probes.

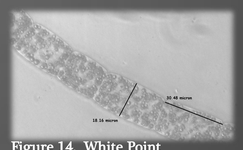


Figure 14. White Point

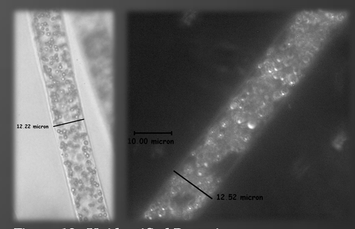


Figure 13. Unidentified Bacterium

Acknowledgements:

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