Metaproteogenomics of the Central Metabolism Utilized by Chemosynthetic Biofilms

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Introduction

Deep-sea hydrothermal vents spew out heated fluids rich in reduced chemical species. Microbial biofilms grow on most surfaces in these resource-rich spots, making them hotbeds of chemosynthetic activity and biodiversity. However, while the phylogenetic diversity of these vent communities is well-known, we understand little about their functional diversity. One factor that can affect functional diversity is the surface on which the biofilm grows: does growth on a living organism significantly affect a vent community? This study aims to reveal the metabolic potential and protein expression of a biofilm on the integument of the giant tubeworm *Riftia pachyptila* using both metagenomics and metaproteomics. A colonizer biofilm in the same bioregime was also collected and sequenced to better contrast with the mature, tubeworm integument biofilm. The samples were investigated for key enzymes indicative of central metabolic pathways as well as the bacteria responsible for these metabolisms.

Sample Name	Sample Site	Age	Temp (°C)	Sulfide Concentration (uM)	pH
CV 88	Crab Spa	Young	15.4	14.4	6.8
Riftia	Riftia	Established	6.6	22.9	7.0

Table 1. The physiological conditions of the sample biofilms. CV 88 is a young, colonizer biofilm while Riftia is the mature biofilm on a tubeworm surface. CV 88 was also closer to the vent fluids and thus in a more reduced environment than the Riftia biofilm.

Methodology

Both samples were taken in the East Pacific Rise (9.8441, -104.2967). The young biofilm (CV 88) was collected using a colonizer mesh while the established biofilm (Riftia) was taken from scrapings off the integument on the base of a *R. pachyptila* tubeworm.

DNA was extracted using a phenol-chloroform protocol. The metagenomes were sequenced with Illumina High Throughput Sequencing and assembled using MegaHit. They were annotated using the JGI IMG/M system, then normalized to transcripts per million (TPM) using a modified Bowtie 2 manual. To obtain their metaproteomes, samples also underwent MS/MS mass spectrometry. Results were processed using X!Tandem software and normalized to percentages in Protein Abundance Index (PAI). Key enzymes indicative of metabolic pathways for carbon, nitrogen, sulfur, oxygen, hydrogen, metals, methane, and halogenated compounds were identified in the metagenomes and metaproteomes.

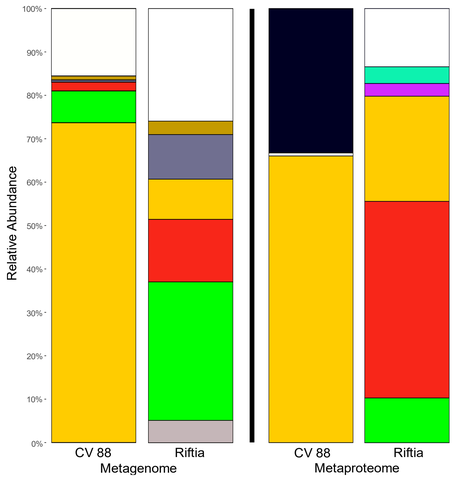
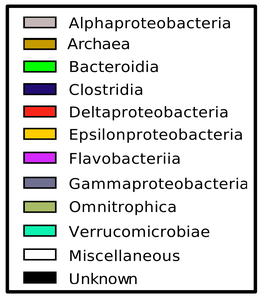


Fig 1. Comparison of the taxonomic composition of the two metagenomes and metaproteomes.



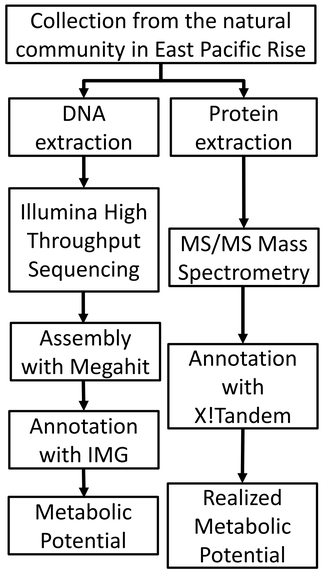


Fig 2. Representation of metagenomic and metaproteomic work.

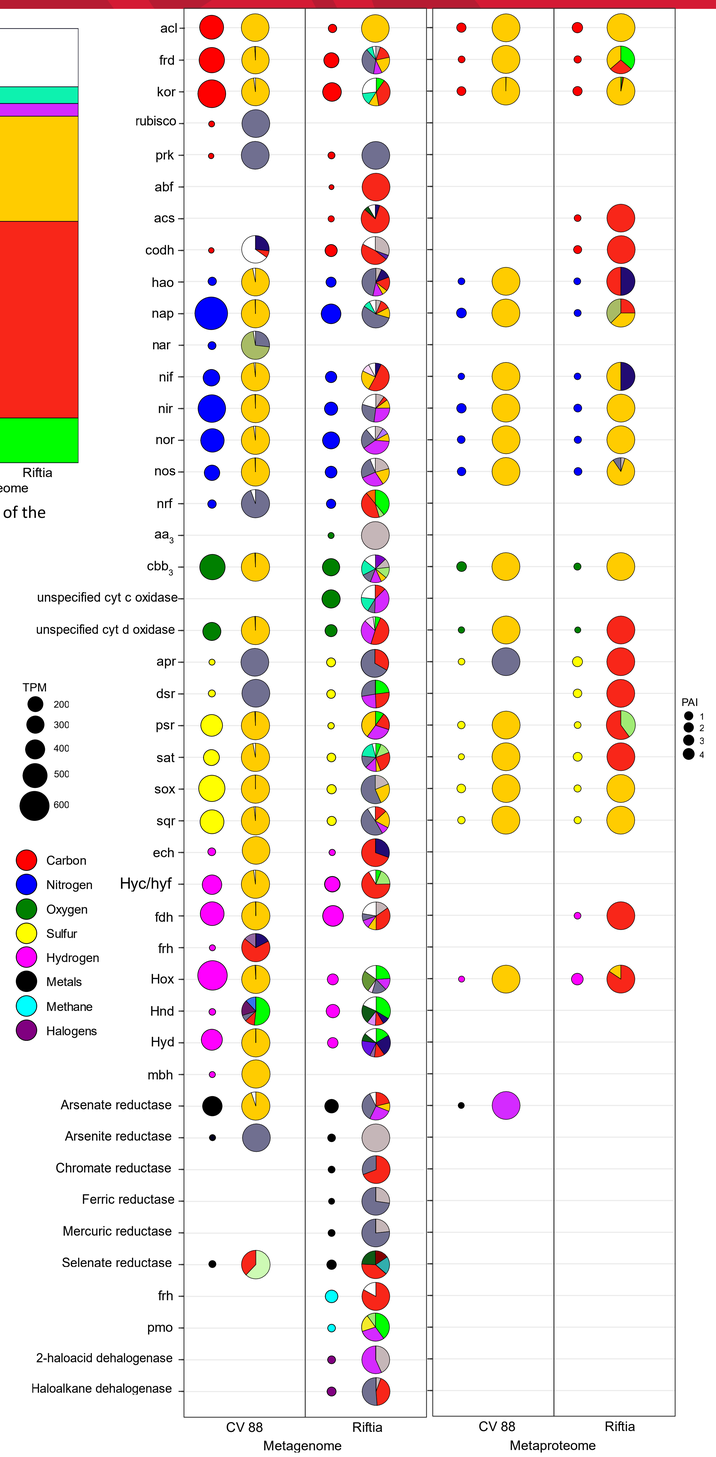


Fig 3. Relative abundances of key enzymes indicative of metabolic pathways and their phylogenetic breakdown. Left: Metagenome in transcripts per million (TPM). Right: Metaproteome in protein abundance index (PAI).

Results and Discussion

Several factors affected the biofilms: age, distance from the vent fluid source, which dictates their exposure to vent fluids, and the surface on which the biofilms grew. Results show an increase in phylogenetic diversity and potential metabolic diversity as the biofilm matured. As the dominant population in CV 88, *Epsilonproteobacteria* acted as primary colonizers due to their metabolic flexibility, fixing carbon with the rTCA cycle and respiring nitrogen, oxygen, and sulfur. Because of both the biofilm matrix and crevices in the tubeworm integument allowing for oxic/anoxic interfaces and thus more available niches, other taxa readily established themselves in Riftia. For example, *Deltaproteobacteria* utilized the reductive acetyl-CoA cycle and became the primary sulfur oxidizers. The more abundant phylogenetic diversity in Riftia also allowed for an increase in the sample's potential metabolic diversity. Despite this increase, both biofilms expressed the same main metabolic pathways, albeit at different concentrations and by different bacteria. One major difference was a significant decrease in carbon fixation genes in Riftia; this is likely because the integument of *R. pachyptila* is made of a chitin proteoglycan-protein complex which acted as an easily accessible carbon source. This is supported by the notable increase in metabolic activity from *Flavobacteria*, which express degradative enzymes like chitinases and proteases, and *Verrucomicrobiae*, which ferment simple organic compounds. Interestingly, the tubeworm's endosymbiont was absent from both the young and mature biofilm. It is possible that there are unknown microbial interactions that discourage its colonization.

Future Direction

Aside from comparisons with other diffuse flow biofilms, there are a multitude of questions to consider. Other adaptations to environmental fluctuations in hydrothermal vents must be investigated, such as flagellar assembly, stress genes, chemotaxis, and DNA repair. Since horizontal gene transfer is a common occurrence in biofilms, proteins for competence, recombination, and transposition must be researched. To confirm that the community is taking up organic material from *R. pachyptila*, it is necessary to explore whether the community utilizes nutrient transporters. Finally, there remains a large knowledge gap in the relationship between *R. pachyptila* and the surface biofilm. For example, does the tubeworm secrete anything that might affect the integument biofilm or vice versa?

References and Acknowledgements

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