Is the Delaware River Harboring Novel Chemolithoheterotrophic Microbes?

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Abstract

Nutrient cycling is a key process for maintaining environmental quality. Microbes drive the flow of nutrients through the carbon and sulfur cycles in both terrestrial and aquatic environments. This project seeks to understand if there are novel microbes in a familiar environment, the local Delaware River, that could link the carbon and sulfur cycles. Both Cultivation-Dependent and Cultivation-Independent approaches are being used to address this issue. Cultivation-Dependent experiments are focused on quantifying and isolating microbes that degrade organic carbon and can oxidize an inorganic compound, thiosulfate, for additional energy. These are called chemolithoheterotrophic microbes. Cultivation-Independent experiments are focused on understanding what microbes were present at the time the river was sampled. Integrating this data will give greater insights into whether chemolithoheterotrophs play an important role in the Delaware River microbial ecosystem. Thus, providing model organisms to further understand the role of these microbes in cycling both carbon and sulfur.

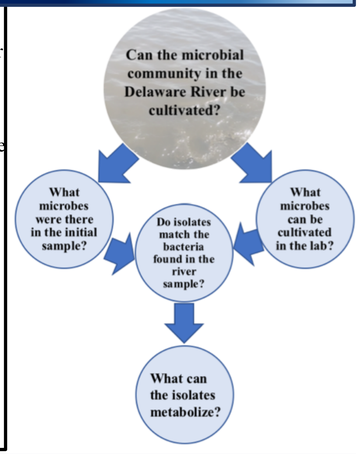
Introduction

To Cultivate Microbes or Not?

- **Traditional cultivation methods** only recover a fraction of cells that can be counted in an environmental sample – the Great Plate Count Anomaly.
- Thus, **Cultivation-Independent** methods were developed to identify microbes in environmental samples.
- **Modern cultivation methods** incorporating signaling compounds and supplemental substrates (nutrient sources) have been developed.

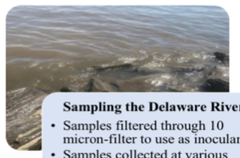
Central Questions

1. Do modern cultivation methods recover representative Delaware River bacteria?
2. Do Delaware River bacteria using organic carbon get extra energy by degrading inorganic compounds?

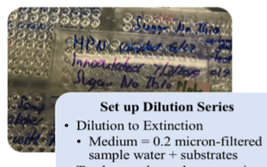


Methods

Cultivation-Dependent



Sampling the Delaware River
- Samples filtered through 10 micron-filter to use as inoculant
- Samples collected at various times of the year



Set up Dilution Series
- Dilution to Extinction
  - Medium = 0.2 micron-filtered sample water + substrates
- Track growth rate by measuring change in protein concentration over time

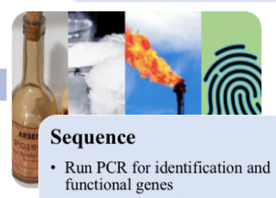
Cultivation-Independent

Sampling the Delaware River
- Sampling time and location same as cultivation dependent
- Preserve unfiltered samples in DESS

DNA Extraction
- Mpbio FastDNA Spin Kit for Soil used for extraction
- Test for DNA concentration using the Qubit reagents

Who? What?

Compare Isolates to Bacteria in Unfiltered Delaware River Water



Sequence
- Run PCR for identification and functional genes
- Prepare samples for sequencing
- Send samples off for sequencing

Results

What Delaware River microbes were cultivated?

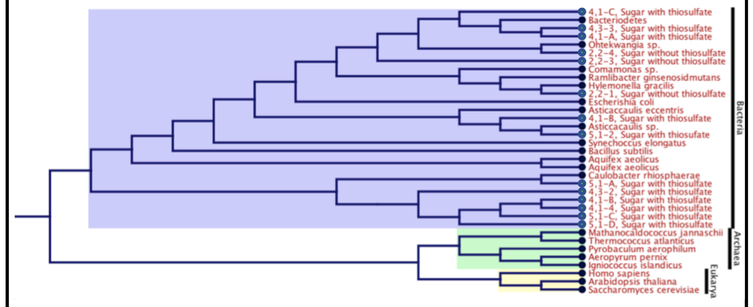
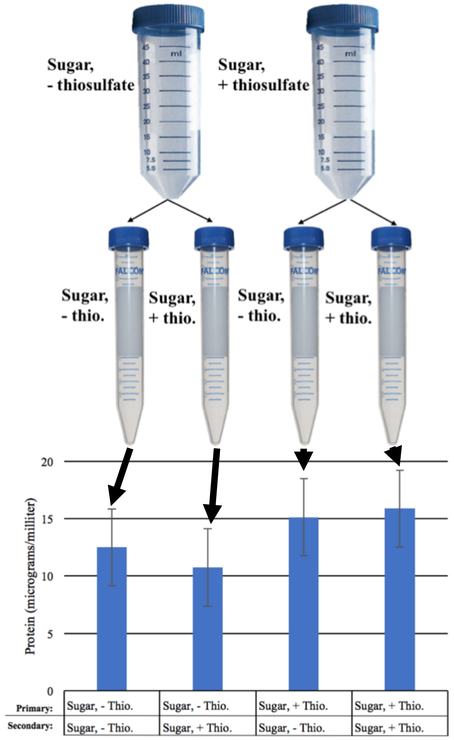


Figure 1: Phylogenetic analysis of the 16S rRNA gene compared to similar and dissimilar sequences. Box color: Blue– Bacteria, Green – Archaea, Yellow – Eukarya. Organisms in the bacteria clade with green circles are samples cultivated in the lab. Samples were sampled on October 18th, 2018.

- Cultures that from the Delaware River are most closely related to bacteria.
- Cultivated bacteria from the Delaware River are Caulobacteri rhizospherae, Asticcacaulis sp., Ohtekwangia sp., and bacteriodetes.

How does growth yield of Delaware River microbes change with addition or removal of thiosulfate?

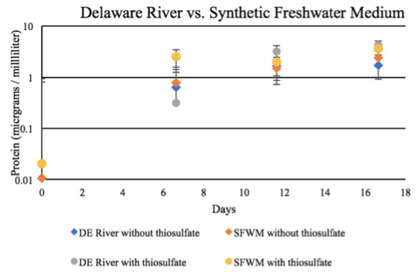


- Growth yield of Delaware river microbes does not change when thiosulfate is added or removed.

Figure 2: Protein analysis of samples from June 27th, 2019 transferred to media containing sources found in the original media with the addition or with the removal of thiosulfate. The x-axis: Primary row = media in which the samples started, Secondary Row = the new media that the starter cultures were added to. Thio. = thiosulfate.

Results Continued

Can Delaware River microbes be grown on synthetic freshwater media?



- Delaware River microbes can grow in synthetic freshwater medium with similar growth rates to Delaware River medium

Figure 3: Protein analysis on samples from the same starting cultures into Delaware River water and synthetic fresh water medium (SFWM).

Conclusions

Cultivation-Dependent

- Bacteria known to live in freshwater environments and in soils can be isolated from the Delaware River.
- When thiosulfate is added or removed, Delaware river microbes grow to the same biomass density.
- The Delaware River microbial community is not dependent on water coming directly from the Delaware River at the time of sampling.
- Synthetic media will yield the same amount of biomass from cultures as cultures grown in the Delaware River.

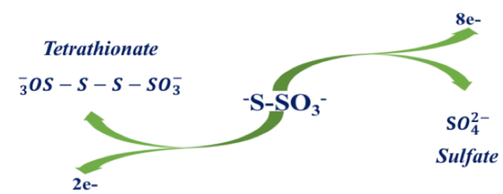
Future Directions

Comparing Isolates to Unfiltered Delaware River Water

- Determine if modern cultivation methods recover bacteria that represent the Delaware River microbial community at the time of sampling.
- Establish what microbes were present at the time of sampling.

Do cultures metabolize thiosulfate and, if so, what do they produce?

- Identify cultures for known thiosulfate oxidizers and evaluate their role in the structure of the Delaware River microbial community.
- Determine if thiosulfate is oxidized into known products, tetrathionate and sulfate, of thiosulfate oxidation.
- Tetrathionate will be measured using high performance liquids chromatography.
- Sulfate will be measured using ion chromatography.
- Identify which pathway for thiosulfate oxidation is preferred by Delaware River microbes.



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