Extracellular Enzyme Activity from Hot Springs in Panama

Microbial degradation of substrates in diverse geothermal features

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Introduction

Microbes found in subsurface water and sediment play an important role in biogeochemical cycling. Heterotrophic microbes require extracellular enzymes to cleave bonds of macromolecules in order to take up and ultimately metabolize organic carbon and nutrients. Geothermal features are characterized by a wide range of temperatures, pH, and other geochemical parameters, and can add to our understanding of microbial extracellular enzyme activities. Here we use different peptidases, polysaccharide hydrolases, and phosphatases as fluorogenic substrate proxies which represent a variety of macromolecules that these enzymes would interact with *in situ*. Our focus here is on extracellular enzyme activities in geothermal features across Panama.

Objective

To better understand microbial degradation of macromolecules in unique geothermal environments

Methods

Microbe-containing syringe filters were collected across Panama at these geothermal features:

Site Name	pH	Temperature (C)
Loz Pozos Thermales (warm)	6.52	39.1
Loz Pozos Thermales (hot)	6.72	55.4
El Salao Campollano	6/7	29.9
Chiguiri Abajo	7	31.1
Los Bajos Corera	7/8	31.8

Methods

These syringe filters were cut into pieces and placed into individual cuvettes containing a citrate-phosphate buffer and one of the 5 following substrates:

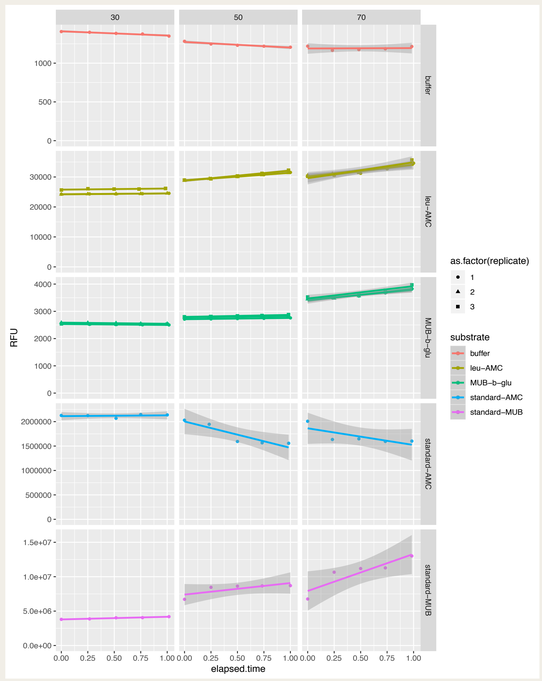
- Leucine-AMC
- Arginine-AMC
- MUB-Sulfate
- MUB-B-Cellobioside
- MUB-PO_4

Cuvettes for one example site shown below (X = control):

Each cuvette was held at the temperature and pH of the respective site. A fluorometer was used to measure relative fluorescence units (RFU) between the control group and the duplicate live samples of each substrate at each site. These measurements were taken every 20 minutes for 2 hours.

Results

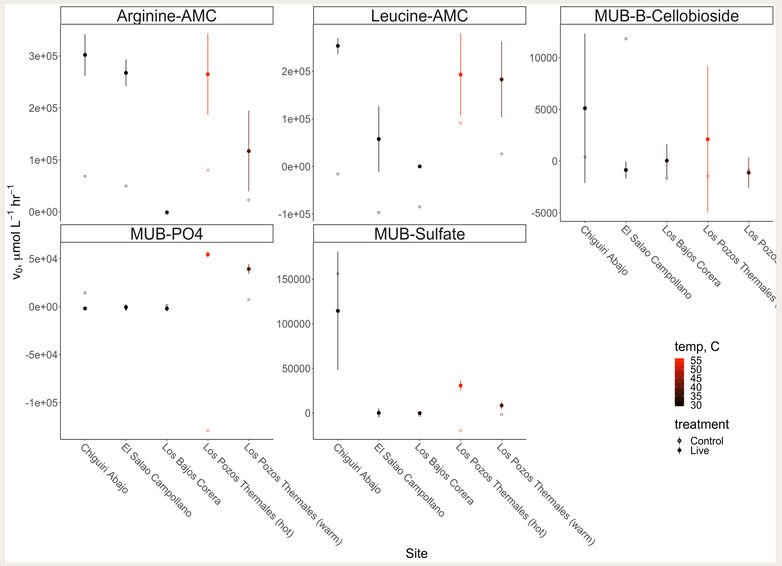
Previous research shows that enzymes are mostly stable when exposed to temperatures similar to those found in these hot springs



References: Schmidt, Jenna Marie, "Microbial Extracellular Enzymes in Marine Sediments: Methods Development and Potential Activities in the Baltic Sea Deep Biosphere. " Master's Cesis, University of Tennessee, 2016. hEps://trace.tennessee.edu/utk_gradthes/4072

Results

RFU activity of Panama live samples over calibrated activity of AMC and MUB standards



- A wide variety of activity is shown depending on the particular site and substrate
- These results offer insight into actual microbial degradation of polymeric material

Note: Due to experimental errors, some control RFU measurements were removed from the raw data.

Conclusions

- Substantial extracellular peptidase activities at all sites
- Low/zero extracellular glycosylase, phosphatase and sulfatase activities (with the exception of Los Pozos)
- Future work: measure the same enzymes in ~7 more sites from Panama and Costa Rica
- Compare these samples to 16S libraries and metagenomes to better understand microbial drivers of organic carbon oxidation in these environments

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