LIFE SIGNATURES AND ORGANIC MATTER PRESERVATION AT THE CATHEDRAL HILL HYDROTHERMAL VENT SITE, GUAYMAS BASIN, GULF OF CALIFORNIA

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Introduction

Guaymas Basin is a submarine depression at the northern end of the East Pacific Rise, mid-oceanic spreading ridge in the Gulf of California. Various hydrothermal vent complexes occur along this margin. One of which is Cathedral Hill, a cluster of white smokers, some encrusted with tube worms (*Riftia pachyptila*) and surrounded by a *Beggiatoa*-dominated sulfur oxidizing microbial mat. Guaymas Basin receives high inputs of organic matter from elevated productivity in the overlying surface waters and runoff from the surrounding continent. The high sedimentation rate produces near-uniform compositions of sedimentary organic matter that is further mixed with the benthic and subsurface micro- and macro-fauna.

In this study we examine the diversity and abundance of polar and apolar compounds extracted from these sediments. This includes intact polar lipids (IPLs) and core lipids (CLs). IPLs are frequently used as biomarkers for living microorganisms in sedimentary environments. Therefore, these compounds can potentially be used to track the habitable range or thermal limits of the subsurface biosphere. By quantifying the distribution of IPLs and CLs we aim to (1) assess the microbial community that inhabits these sediments at a chemotaxonomic level, (2) determine the thermochemical stability of these lipids, and (3) trace the degradation pathways that may result from their pyrolytic conversion into hydrocarbons. We also aim to elucidate how the hydrocarbon matrix is attenuated or added to by biodegradation, thermochemical degradation, *in situ* hydrocarbon production, and migration.

Results

Four push cores (down to 23 cmbsf, 34 samples) were collected along a transect line running from the center sulfide chimney complex to the outside of the microbial mat using HOV *Alvin*. Thermal-probe measurements yielded dramatic increases with depth. By 7-9 cm, pore-fluid temperatures were 105°C in the vent center marking the most shallow expected habitable limit to the microbiome (from 18-122°C). Projected porewater temperatures reach up to 155°C within only 21cmbsf. These higher temperatures should not only be capable of pyrolyzing *in situ* organic matter, but begin to thermochemically break down these newly formed compounds to generate a petroleum-like fluid.

Upon sample collection, push cores were immediately cut into 2 cm-thick sections. The resulting samples were frozen in combusted glass vials at -70°C and lyophilized prior to solvent microwave and Bligh & Dyer extractions. Identified lipids include archaeal IPLs and CLs, such as archaeol (AR, 1G-AR, 2G-AR, 1MeC-AR), glycerol dialkanol diethers (GDDs, OH-GDDs), and gycerol dialkyl glycerol tetraethers (1G- and 2G-GDGTs, iGDGTs, brGDGTs). Also present in the samples are multiple unknown phospholipids that are likely bacterial in origin. Most of the identified compounds have distinct stratigraphic trends. For example, 2G-GDGTs, possibly derived from anaerobic methanotrophs, were extracted from sediments ranging up to ~50°C. In contrast, 1G-GDGTs are observed in sediments reaching ~145°C,

indicating hyperthermophilic archaea at these temperatures. The abundance of core iGDGTs decrease with sediment depth as do GDGTs closer to the vent center. Little to no biphytanes, which are degradation products of GDGTs, are found in these sediments suggesting a kinetic rate limit and more time is needed to crack the lipids into hydrocarbons. However, biphytanes (with one to two cyclopentane rings) are found in trace amounts at 6-10 cmbsf along the low temperature mat fringe and ambient sediments. Here sediment temperatures are too low to promote cleavage of the isoprenoid skeletons and the source of these lipids is yet unclear.

The polar fractions analyzed by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) in electrospray ionization mode, contain an abundant array of acid and neutral compounds. Semiquantitative analysis of Z class ($Z_{number}=C_nH_{2n+z}+H_n$) and molecular weight distributions display systematic depth and distance changes, with acid and diacid functional groups being most resilient in these hydrothermal settings. The apolar hydrocarbon fraction of sediment extracts have been analyzed via a multi-molecular, chemometric survey using comprehensive two-dimensional gas chromatography (GC×GC). Subtracted GC×GC chromatograms reveal elevated levels of high-temperature pyrolytic hydrocarbons, including relatively high abundances of higher molecular weight PAHs (pyrene to coronene) and equivalent perhydro-PAHs, at 6-10 cmbsf consistently across the transect. This band corresponds to a wide range of sediment temperatures spanning 18 to 125°C, which for the outer perimeter of the vent is too low for active *in situ* pyrolysis. Elevated CPI values indicative of higher plant wax contributions occur as four discrete patches spanning 7 to 21 cmbsf within the push core transect. Hierarchical cluster analyses of whole GC×GC stacked chromatogram indicate hydrocarbon variability forms a patchy profile of overprinting to stratigraphic and vent flow patterns. Collectively, these data indicate that oil primarily derived from multiple charge events deeper within the basin. An unknown pseudohomologous series of tetracyclic compounds, along with decoupled ratios of bacterial-sourced lipids C_{30} hopene and C_{30} hopane (indicating the hopene biomarker is not being thermal altered to hopane) implies that a living bacterial community is hosted at shallower sediment depths in the vent complex. Lastly, ratios of low to intermediate molecular weight *n*-alkanes and acyclic isoprenoids show increasing levels of biodegradation (reaching 2-3 on the Wegner et al., 2002, biodegradation scale) down-core and across the transect suggesting some metabolic specificity to the microbiome.

Conclusions

Currently the most extreme thermophile is Strain 121 *Geogemma barossii*, a lithoautotrophic archaeon isolated from an active black smoker. *Geogemma barossii* can grow and reproduce at 122 °C and remain biostatic for 2 hrs at 130 °C (Kashefi and Lovley, 2003). However, Holden and Daniel (2013) speculated that the actual thermal limit for microbial growth may be as high as 140 °C. The lipidomic data from this study suggest that life might indeed exist beyond the limit of current known cultures. These results also suggest that either more time is needed to crack many of the detected lipids into hydrocarbons or that some lipids are more stable and can preserve to become more deeply buried in these pyrolytic environments.

References

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