**Microevolutionary dynamics in *Methanothermococcus* populations from deep-sea hydrothermal vents in the Mid-Cayman Rise**

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Deep-sea hydrothermal vents sustain diverse communities of microorganisms. The effects of physical, geochemical and biological interactions on the process of evolution in these deep-sea ecosystems remains poorly understood because the majority of subsurface microorganisms remain uncultivated. By examining metagenomic samples from hydrothermal fluids and mapping the samples to closely-related genomes found in vent sites, we can better understand how the process of evolution is affected by the geochemical and environmental context in deep-sea vents. The Mid-Cayman Rise is a deep ultra-slow spreading ridge that hosts both mafic-influenced and ultramafic-influenced vent fields. Previous research on metagenomic samples from sites in the Mid-Cayman Rise has shown that these vents contain taxonomically and metabolically diverse communities consistent with the diversity of metabolic compounds generated by the mafic and ultramafic vents’ geochemical activity. Here, we investigate five single cell amplified *Methanothermococcus* genomes (SAGs) to investigate patterns in pangenomic variation and molecular evolution in these methanogens. Mappings of metagenomic reads from 15 sample sites to the SAGs reveal substantial variation in *Methanothermococcus* population abundance, nucleotide variability and selection pressure among the 15 geochemically distinct sample sites. Within each sample site, we observed distinct patterns of single nucleotide variant (SNV) accumulation and selection pressure within the populations represented by each of the SAGs. Genomes that were more closely related to each other showed similar patterns of SNV accumulation. Analysis of open reading frames (ORFs) from the SAGs indicated that homologous genes with similar function accumulated variation at the same rate. For example, a genomic island containing genes involved in nitrogen fixation was identified in three of the five genomes with significantly elevated SNV counts. dN/dS analyses revealed evidence for frequency-dependent selection, in which genes unique to individual SAGs displayed elevated diversifying selection relative to other genes. These results indicate that different strains of *Methanothermococcus* outcompete others in specific environmental settings, and that these fitness advantages may result from variation in the pangenome, as revealed by the dN/dS and SNV analyses. By examining variation and the scale of nucleotide and genes, we aim to gain insight into the roles of genetic diversity and environmental selection on microbial evolution in these ecosystems.