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Title: Viruses of Earth's Continental Deep Subsurface: Analog Study of a Potential Biomarker for Extraterrestrial Life

Abstract:

Viruses are the most abundant biological entities on Earth. However, to date, almost all studies of the deep biosphere have focused on cellular life and almost nothing is known concerning free viral particles. Here we propose the genomic analysis of subsurface RNA and DNA-based viruses obtained from a borehole, Inyo-BLM 1 (BLM-1), which intersects upwelling geothermal fluids of the Death Valley Regional Flow System. The initial phases of the proposed study are already completed, setting the stage for timely implementation of the proposed task and a high probability of success. Specifically, viral preparations from deep groundwater were recently concentrated using a method developed in our lab for SARS-CoV-2 surveillance in wastewater; which resulted in measurable quantities of pure viral DNA and complementary DNA (cDNA; DNA synthesized from RNA). This material is now ready for analysis and represents the starting point for the proposed work. If funded, our next stage will be to sequence viral DNA and cDNA and use newly developed metagenomic pipelines to resolve taxonomic and evolutionary relationships. Evidence of prior viral integration into bacterial genomes from BLM-1 will be assessed with particular attention to Clustered Regularly Interspersed Palindromic Repeats (CRISPRs), an antiviral defense system of prokaryotes. The new capacity to analyze subsurface viral genomes in our lab is based on an ongoing viral survey from lava caves in close collaboration with our NASA collaborator and co-I of this proposal, Dr. Jennifer Blank, of NASA Ames. Our BLM-1 samples likely represent the most complete virome ever obtained from a continental deep subsurface system and thus may be a unique opportunity to examine mobile genetic elements and their genomic footprints in one of the least understood, but most relevant and highest quality analogs for life on celestial bodies.