Cultivable halophiles from cold geysers as indicators of a deep subsurface habitat

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Introduction: The goal of this project is to assess the microbial diversity of two cold geysers in Utah (Crystal Geyser (CG) and Ten Mile Geyser (TMG)) and to search for potentially novel halophiles in the hypersaline deep aquifers that underlie these geysers. Halophiles can tolerate many forms of environmental stress, and their general stress response systems can be informative for understanding the constraints for life on Mars and other planets. Moreover, prokaryotic rhodopsins were discovered in halophilic archaea, and retinal-based phototrophy may be one of the oldest metabolic capabilities on Earth [1]. Hence, these extremophiles can be useful models for studying the early evolution of metabolism.

Both geysers are located on the east bank of Green River, Utah. They are cold-water, carbon-dioxide-driven geysers, and they are surrounded by travertine deposits. The eruption of these geysers is similar to hot-water geysers, except that CO_2 bubbles take the place of steam [2]. The two main aquifers that supply water to these geysers are located at different depths and in different rock layers. The first one is in sandstone at a depth of 300-500 m. It supplies 80-90% of the eruption water, and its salinity is quite low. The other aquifer is in a saturated salt rock layer at a depth of 1500 m. It supplies 10-20% of eruption water [3][4][5].

Methods: We measured the temperature, pH, and conductivity of the geyser eruption waters, and based on these results we designed the pH of the medium and incubation temperature for culturing of halophiles on agar plates. We chose a low substrate concentration medium (NSY) to allow slow-growing microbes to use a limited source of organic carbon and energy for their growth [6]. We prepared several replicates of this medium with varying salinities. Because the geyser water has relatively low biomass, we concentrated cells by centrifugation and plated centrifuged cell pellets as well as non-centrifuged samples. Growth was observed within 72 hours of incubation, and then colonies were selected from the source plates and cultured on agar plates with salinities ranging from 2-25%. Subculturing of the colonies is now underway.

Expected Results: If these potentially halophilic strains can grow at salinities that resemble the conditions of the hypersaline deep aquifer, then this will be strong evidence of a potentially active halophilic community in the deep subsurface. Future work will include checking the microbial colonies of each plate under the microscope to make sure that they are purified and to characterize their morphological features. Finally, we will sequence the 16S rRNA genes and then the genomes of any potentially novel halophiles to compare to known halophiles and other subsurface organisms. These results will inform efforts to conduct a census of subsurface halophilic communities and to characterize the biological constraints of subsurface hypersaline habitats.

Key words: halophiles, cold geysers, aquifer, subsurface

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