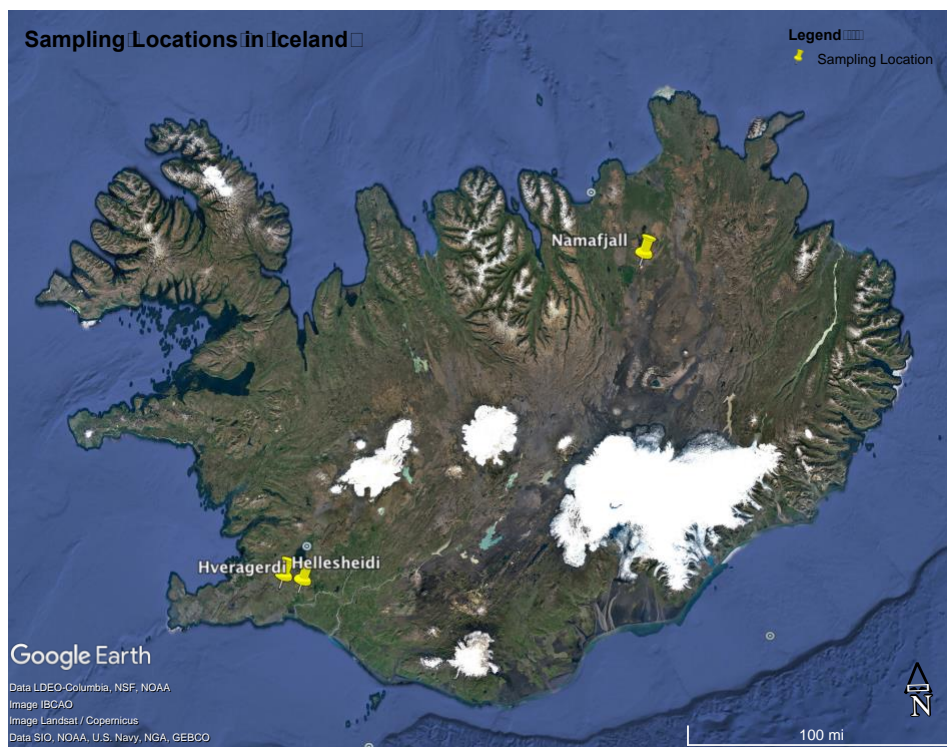


**“QUANTIFYING THE INFLUENCE OF TECTONIC REGIME ON HOT SPRING MICROBIAL DIVERSITY”  
Project Report**

**Overview of project.** The funds requested in this proposal were allocated towards costs of travel to Iceland in order to sample hot springs across several regions. Microbial community compositional analyses of the samples will be conducted and compared against corresponding in-house datasets that are available for Yellowstone National Park (YNP) hot springs, as well as now published datasets for hot springs in the Taupo Volcanic Zone (TVZ) of New Zealand. The aim of the overall project is to investigate the role of differing tectonic and geologic contexts on the composition and function of chemosynthetic microbial communities inhabiting high temperature hot springs among globally distributed, geologically distinct, continental hydrothermal environments.

Field work was conducted in late summer (August 23<sup>rd</sup> through September 5<sup>th</sup>) of 2018 in collaboration with Dr. Andri Stefánsson and graduate students from his laboratory at the University of Iceland in Reykjavik. Dr. Stefánsson is a professor in geochemistry with several decades of experience researching the geology and geochemistry of geothermal systems of Iceland. In collaboration with his group, field work was conducted in northeastern Iceland (in the Námafjall region: N 65.64277 W 016.84118; Fig. 1) as well as in southwestern Iceland (in the Hveragerði: N 64.00782, W 021.18030 and Hellesheiði: N 64.01992 W 021.39385 areas; Fig. 1).



**Fig. 1. Map of Iceland showing the three regions that were sampled as part of the present project.**

**“QUANTIFYING THE INFLUENCE OF TECTONIC REGIME ON HOT SPRING MICROBIAL DIVERSITY”  
Project Report**

**Description of Sampling Efforts.** Sampling was conducted to maximize the range of geochemical conditions that would be generally expected among hydrothermal systems. The predominant geochemical factors that control microbial population distributions in hydrothermal systems are temperature and pH, and thus, sampling was conducted among springs exhibiting a wide range of pH (2.45 – 9.75) and temperature (50 – 95°C) at the three sampling regions. Sampling was preferentially conducted on springs that exhibited similar temperature and pH profiles as is observed in YNP springs, but with clearly different geochemistry based on visual inspection of waters and associated sediments. In addition, where possible, borehole discharge fluids were sampled that represented deeply-sourced fluids that have had little contact with rocks upon ascension to the surface. Both planktonic (water-associated) microbial communities as well as sediment-associated communities were sampled from 22 different geothermal features, totaling 44 microbial community samples, with triplicate samples taken for sediment samples. A general description of the nature of samples from each region is described below.

**Námafjall.** The area around Námafjall is characterized as a typical acid-sulfate system, with considerable amounts of geothermal steam (carrying volcanically derived gases) that causes significant amounts of surface alteration. In addition, several geothermal power plants are located in the area, with associated boreholes accessing deep hydrothermal fluids. The area that was sampled was a small acid-sulfate drainage with several acidic, high-temperature mud pots (Fig. 2), in addition to borehole fluids from three different pipelines (Fig. 2). A larger, different geothermal field that is a highly visited tourist area can be found a few kilometers to the east.



**Fig. 2. Two of the sampled geothermal areas at Námafjall. Left: acidic (pH < 3) mudpots within a small acidic geothermal field. Right: hydrothermal borehole fluids (T~85°C) draining into an artificial geothermal creek.**

**“QUANTIFYING THE INFLUENCE OF TECTONIC REGIME ON HOT SPRING MICROBIAL DIVERSITY”  
Project Report**

Of particular interest were the borehole fluids which allowed access to geothermal waters that would have had minimal interaction with surface-associated fluids, and would thus be highly insightful towards understanding the potential for native, deep hydrothermal biospheres in subsurface hydrothermal fluids. These samples are particularly valuable, as such types of environments are currently not possible to access in Yellowstone National Park, where drilling is not allowed and previously drilled wells are not currently accessible. Further, the geothermal fluids exhibited high pH values, even upwards to  $\text{pH} \sim 10$  that are anomalous for an acid sulfate area, and for hydrothermal fluids, in general.

**Hveragerði.** As in Námafjall, Hveragerði is a predominantly acid-sulfate type geothermal field with extensive geothermal activity throughout multiple drainages. The area surrounding the town of Hveragerði features numerous geothermal fields and is in general, a highly active geothermal region. Thus, the area was used to collect additional, primarily low pH samples from springs and mudpots that were atypical in character to those found in YNP (Fig. 3).



**Fig. 3. Acidic ( $\text{pH} < 5$ ) geothermal features in the Hveragerði region of southwestern Iceland. Left: graduate student field assistant from the University of Iceland sampling a small iron-rich acidic spring. Right: large weakly acidic mudpot that was sampled.**

**Hellesheidi.** The area referred to as Hellesheidi was also located in southwestern Iceland,  $\sim 15$  km west of Hveragerði. Unlike the other two geothermal sites that were sampled, Hellesheidi exhibited a wide diversity of spring types, with numerous acid-sulfate type springs as described above, in addition to a multitude of circumneutral springs that were atypical for circumneutral geothermal waters as observed in geothermal systems like Yellowstone. Many of the springs were evolving considerable amounts of gas, which is likely to influence the character of both acidic and circumneutral springs exposed to such gas. The influence of gas is noted by dark black/grey sediments in circumneutral features that are likely due to pyrite minerals formed via

**“QUANTIFYING THE INFLUENCE OF TECTONIC REGIME ON HOT SPRING MICROBIAL DIVERSITY”  
Project Report**

large amounts of H<sub>2</sub>S degassing (Fig. 4) – a phenomenon that is rarely found in geothermal fields of YNP.



**Fig. 4. Circumneutral (pH ~ 7) geothermal features in the Hellesheidi region of southwestern Iceland. Left: spring evolving considerable gas (as seen by visible degassing), and black/grey sediments throughout the spring. Right: a geochemically similar spring as that on the left, exhibiting dark sediments throughout the spring and outflow.**

**Planned future analyses and integration with existing datasets.** The microbial diversity associated with the samples collected from Iceland will be investigated using 16S rRNA gene sequencing. Community composition will then be compared against that of springs with similar geochemistry in YNP and from the TVZ. These data will be used as preliminary data for a follow on proposal that is currently being prepared for submission to NASA’s EPSCoR program through the Montana Space Grant Consortium. In addition, a selection of samples from Iceland will be subjected to total community shotgun genomic sequencing and reconstruction of individual population genomes to infer the metabolic functional capacity of populations within Icelandic springs that could be compared against similar datasets that exist for YNP springs. These data will be collectively synthesized in order to address the primary hypothesis of this work regarding the role of tectonic regime on hydrothermal geochemistry, and subsequently, the nature of microbial populations that are allowed to dwell in these habitats across Earth.