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Lewis and Clark Fund for Exploration and Field Research in Astrobiology

"Single-cell genome sequencing of a novel *Gloeobacter* from an epilithic biofilm in a Hawaiian basaltic cave"

Project Report/Summary

1. Field Trip details

Expedition personnel:
Jimmy Saw, Stuart Donachie, and Keali`imanauluokeahi Taylor (field guide).

The field trip to Kilauea Caldera in Hawaii Volcanoes National Park in Volcano took place on October 31, 2009. The caldera was and remains closed to pedestrian and vehicular traffic due to ongoing volcanic activity. However, our permit allowed us to enter the caldera by car along the Crater Rim Driver to what was a parking lot close to Halema'uma'u pit crater. The entire visitors' area has been destroyed by pyroclastic fragments, so we left our vehicle further from the pit crater than the furthest flung boulder. We walked from the car to the sampling site, according to GPS data from a previous visit, a distance of about 2 km and which took about one hour.
The sampling site is a lava cave referred to as Big Ell. GPS coordinates are approximately 19.425154, -155.274582 (see green arrow in Fig. 1A, and yellow arrow in Fig. 1B). The cave is located in the 1919 lava flow. Entry to the cave is through a ground level entrance of ~1 x 0.5 m (Fig. 2B). Part of the ceiling extends immediately below the entrance but is not directly illuminated. Conditions in the cave may be considered extreme, with relative humidity over 100%, air temperatures of 30-40ºC, and a persistent wind sweeping from within the cave as warm air rises from within and exits through the entrance. Heavy condensation formed on surfaces in the cave. Parts of the cave floor at depths of ~3cm have reached 90°C (measured by temperature probe).

Fig. 1. (A) Approximate location of Big Ell cave in Kilauea Caldera. Cave location shown by green arrow. **(B)** Sampling site in Kilauea Caldera. **(C)** Steam rises from Halemaʻumaʻu pit crater. **(D)** Moving towards the sampling site.

Care was taken to sample the purple biofilm in the cave's entrance without removing all trace. For example, since we first observed the biofilm in 2006, the relative area of the purple material thought to contain mostly *Gloeobacter* had shrunk from ~50% to just a few percent by 2009. That said, regardless of which color of material was on the surface, the biofilm did seem to be a mixture of both green and purple cells (Figs. 2C, 2D).

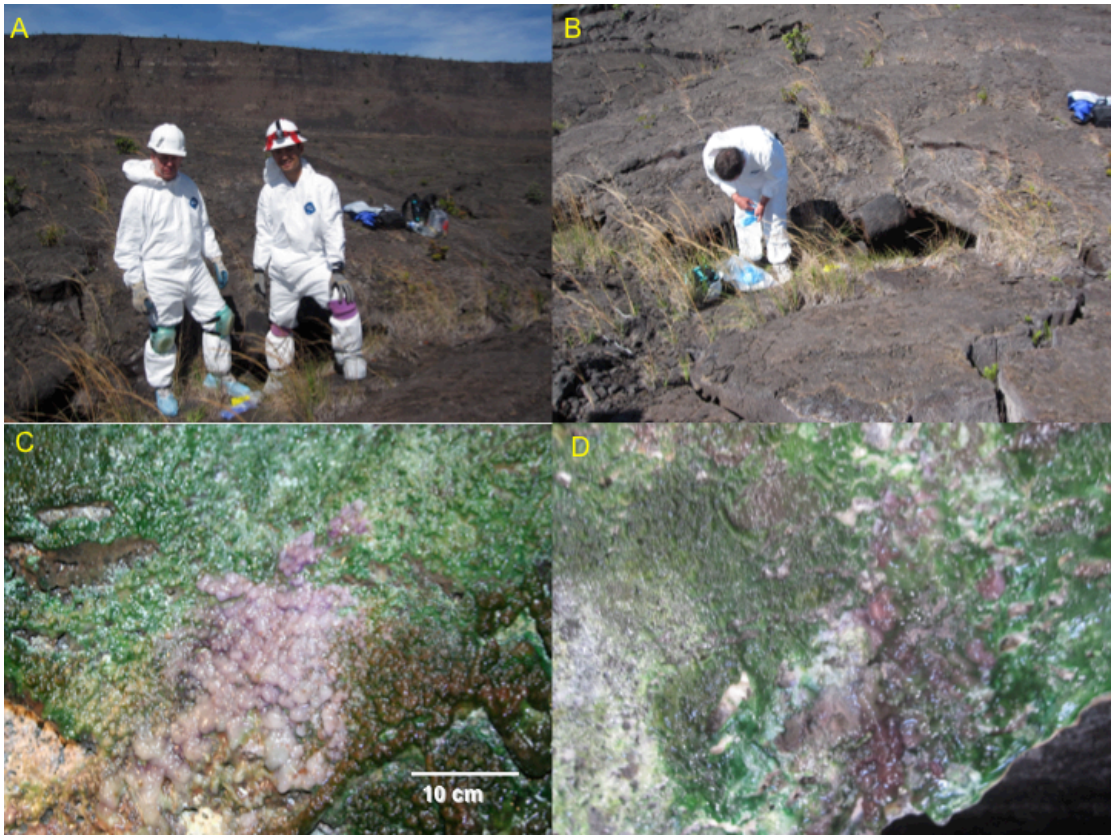

Figure 2. (A) Stuart Donachie (L) and Jimmy Saw before entering Big Ell cave. **(B)** Stuart Donachie in front of the Big Ell entrance. **(C)** Biofilm in 2006. **(D)** Biofilm in 2009.

Ten samples were collected from the purple area of the biofilm into six 2ml cryotubes (two without preservative, two with RNAlater, two with fixative for electron microscopy), and four 15 ml polypropylene tubes containing different cultivation media.

2. Single-cell isolation work

Upon return to the laboratory, 1-2 mm^3 of purple biofilm was vortexed in a 1.5 ml microfuge tube. The resulting cell suspension was dissected in a laser tweezers system to isolate single-cells. Single-cells were removed from the material by laser catapulting into the caps of 0.2 ml microfuge tubes. These cells were lysed and their whole genome amplified in the GE GenomiPhi V2 DNA amplification kit. This resulted in single-cell amplified genomes (SAGs). Amplified genomic DNA was then used as the template from which fragments of the 16S rRNA gene(s) were amplified to confirm selection of the target *Gloeobacter*. This approach did not yield the best product from which to subsequently sequence the *Gloeobacter* genome, and contamination issues hampered workflow. Encouraging results from the cultivation experiments led to a change in experimental plans, with a move to a cultivation-based approach.

3. Cultivation work

The initial plan was to use a single-cell genomics approach for the novel *Gloeobacter* in the biofilm. However, being able to provide a culture of the new species was thought to be of potential value to researchers in the field of cyanobacteria. Biofilm material was thus spread on a defined medium designed to select for cyanobacteria such as *Gloeobacter*. Using a modified BG11 medium and low light levels, we cultivated the new *Gloeobacter*; the strain was designated JS1 (Fig. 3). Sequencing of the 16S rDNA sequence amplified from the genomic DNA of JS1 confirmed this part of the strain matched that in the uncultured clone from our previous sampling (Fig. 4).

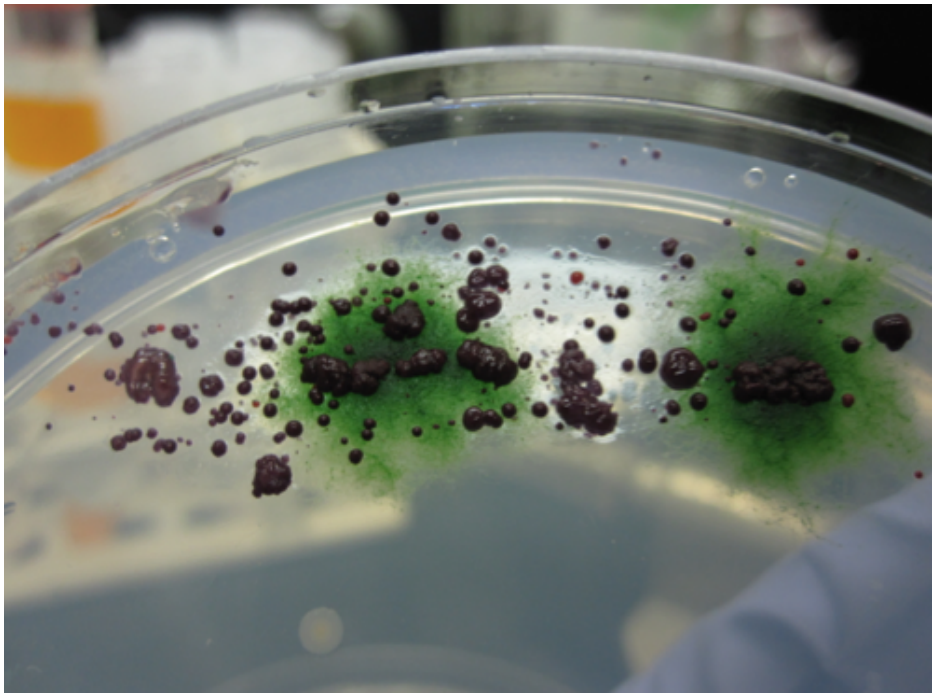

Fig. 3. Non-axenic *Gloeobacter* JS1 (dark, elevated colonies) on modified BG11 agar.

4. Deposit of *Gloeobacter kilauensis* in international culture collections

Strain JS1^{T} has been deposited in the American Type Culture Collection (BAA-2537), the Scottish Marine Institute's Culture Collection of Algae and Protozoa (CCAP 1431/1), and the Belgian Coordinated Collections of Microorganisms (ULC0316). The *G. kilaueensis* holotype has been deposited in the Algal Collection of the US National Herbarium (US# 217948).

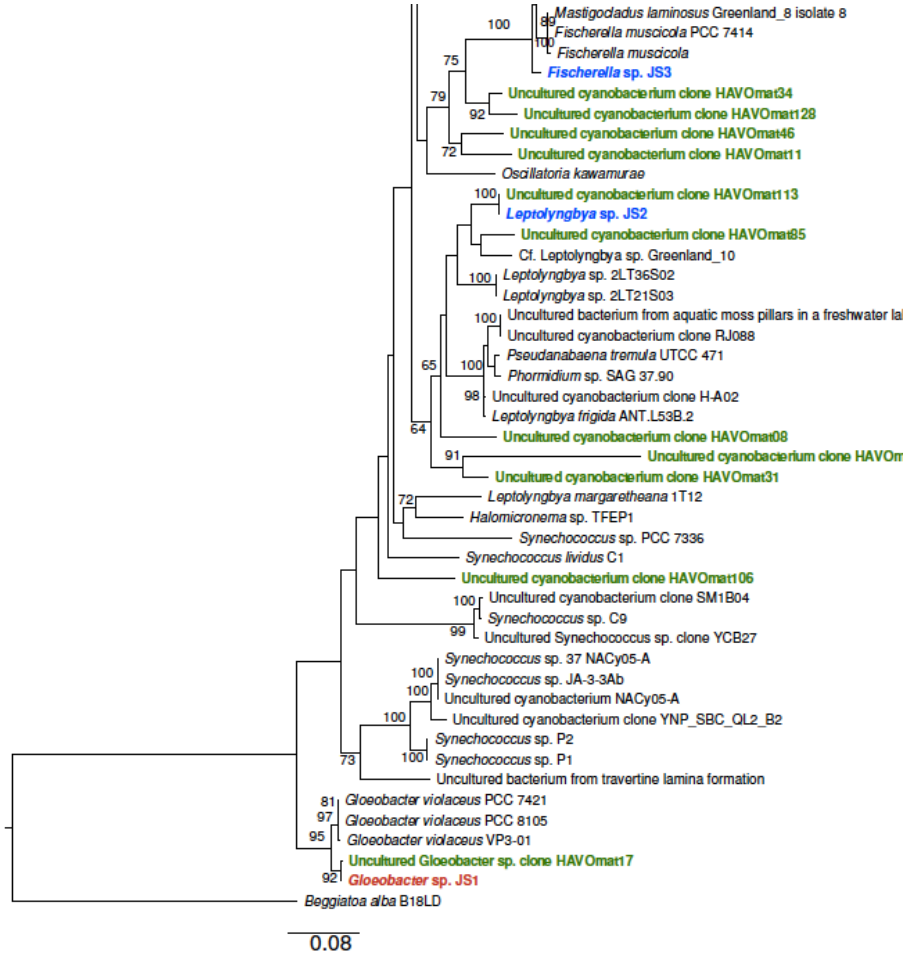

Fig. 4. Maximum likelihood phylogenetic tree of cultivated cyanobacteria from the cave biofilm. JS1, highlighted in red, perfectly matches a clone from our previous collection work in the cave.

5. Genome sequencing and analysis

While we succeeded in cultivating a new species of *Gloeobacter*, obtaining an axenic culture from which to extract DNA was difficult in the time available. We thus prepared a sequencing library from an enriched culture, one containing predominantly the targeted *Gloeobacter* cells, but also cells of other bacteria. Next-generation sequencing (NGS) data were generated from this library through 454 and Illumina sequencers, and assembled with the Celera Genome Assembler. These sequence data enabled assembly of the complete genome sequence of JS1. Our results confirmed that the *Gloeobacter* isolated from the cave is a new species, for which we proposed the name *Gloeobacter kilaueensis*. This is only the second member of the genus, and the first description of a new *Gloeobacter* in almost 40 years. The detailed findings have been accepted for publication in PLoS ONE. The complete genome sequence of JS1^T has been deposited in GenBank under accession number CP003587.

6. Publication from this research

Jimmy H. W. Saw, Michael Schatz, Mark V. Brown, Dennis D. Kunkel, Jamie S. Foster, Harry Shick, Stephanie Christensen, Shaobin Hou, Xuehua Wan, Stuart P. Donachie. Cultivation and complete genome sequencing of *Gloeobacter kilaueensis* sp. nov., from a lava cave in Kīlauea Caldera, Hawai‘i. PLoS ONE. DOI: 10.1371/journal.pone.0076376