Identifying Caltech campus horticulture and microbial fuel cell communities by 16S rRNA barcoding

Cadence Dam[1], Dante Dam[1], Lucas Garcia[1], Matteo Ornelas[1], Ray Chung[1], Troy Reyes[2], Jael Santos[2], Monica Barsever[1], Alec Lourenço[3]

[1]Institute for Educational Advancement, [2]Benjamin Franklin High School, [3]California Institute of Technology

Contents

1 Abstract

This study characterizes the bacterial community generating electricity in our microbial fuel cells by targeting the 16S rRNA gene for PCR, revealing a diverse collection of electrogenic bacteria known in literature. We also sequence and identify plant DNA yielded by applying the same procedure to a *Monomorium minimum* (little black ant) collected at Caltech. We compare the resulting plant DNA to a map of trees on campus.

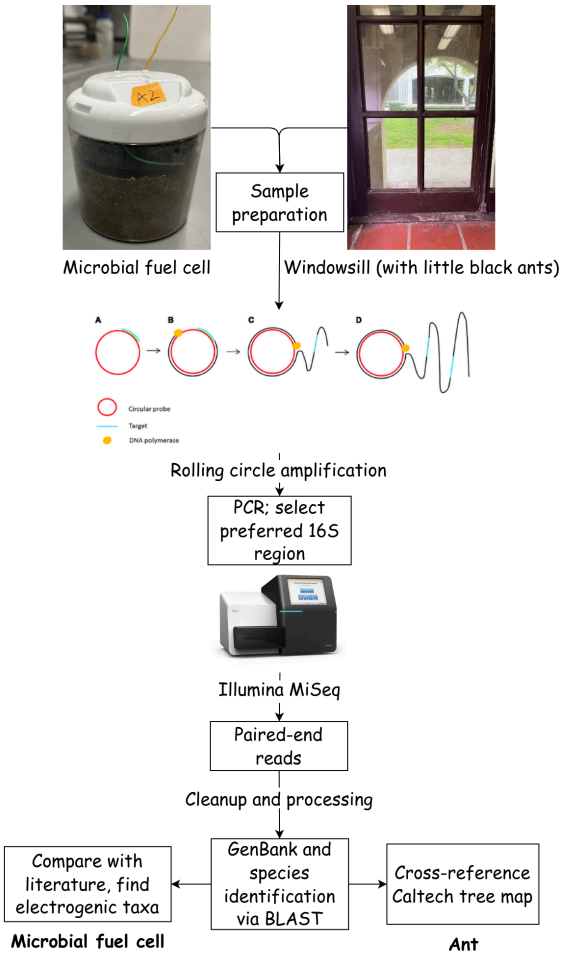

Figure 1: An overview of our pipeline in this study.

2 Background

Microbial fuel cells (MFCs) harness microbes to produce electricity from organic substrates. Electrogenic bacteria in anaerobic conditions metabolize a substrate and release electrons, creating a usable potential difference [1]. One such reaction which is known to occur on our fuel cells is detailed below:

$$\text{Anode: } \mathrm{CH_3COO^- + 2H_2O \xrightarrow{bacteria} 2CO_2 + 7H^+ + 8e^-}$$

$$\text{Cathode: } \mathrm{2O_2(from\ air) + 8e^- + 8H^+ \longrightarrow 2H_2O}$$

Many other reactions are of course possible on a range of organic substrates, including sugars [2].

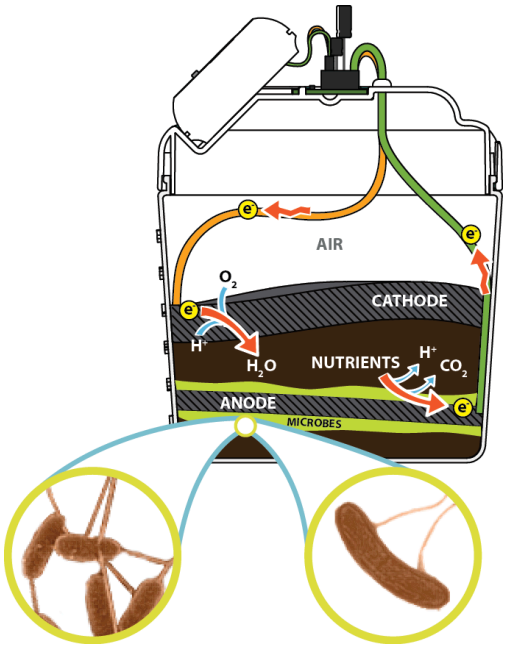

Figure 2: A diagram of a microbial fuel cell similar to ours [3].

2.1 Motivation

In their current state, microbial fuel cells are not suitable for commercial grid power generation. But they may be useful in applications which require small voltages, or in a wastewater treatment with power generation as a side product. It has been proposed that the digestion of organic matter at water reclamation plants be handled by microbial fuel cells which would both digest the material and generate electricity [4].

2.2 Prior research

During SEAL 2022-2023, this group investigated our MFCs by "dosing" fuel cells with various quantities of capsaicin via Tobasco sauce and caffeine via OTC caffeine pills [5]. But throughout this experiment we remained uncertain as to the identity of the electrogenic bacteria which were reacting to our pollutants. They were assumed to include *Shewanella oneidensis* and *Geobacter metallireducens*, commonly found in such MFCs. But many others have found different species with similar electrogenic properties [6]–[10].

3 Our work here

Regardless, we sought to identify the microbes responsible for the continued electrical activity in our fuel cells. The organic material in our fuel cells had two parts [5]. One was soil from a home garden in La Crescenta, CA. The other component was a bacterial sludge distributed by the LA County Sanitation Districts (LACSD) for the purpose of

demonstrating biodigestion of organic matter in wastewater, again pointing to the dual potential of MFCs.

3.1 Earlier attempts

We attempted this sequencing several times.

On our first attempt, we sampled the dirt cathode of the cell and received no DNA which could be conclusively traced to any meaningful taxa. We suspect multiple issues were at fault: our Sanger sequencing approach, lab error, issues with primer selection, and the relative lack of bacteria at the cathode compared to the anode (we intended to sample the anode but simply forgot). A DNA electrophoresis was performed with a 1% agarose gel and E-Gel 1Kb DNA Ladder (Thermo Fisher Scientific). The presence of DNA in the electrophoresis below yet lack of resolution from sequencing suggests Sanger sequencing was inadequate (as would be expected when sequencing a diverse community).

A *E. coli* control was successfully implemented for this step and returned blastn [11] hits for *E. coli* when sequenced, again suggesting Sanger sequencing was inadequate.

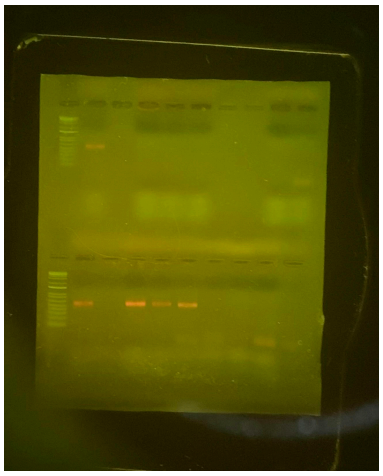

Figure 3: Electrophoresis from our October sequencing attempt. The ladder places these reads around 350bp (the expected length of PCR product in *Shewanella*) but Sanger sequencing was unsuccessful.

On our second and third attempts, we moved to the anode of the cell and began using the Illumina MiSeq platform.

4 Successful attempts

While carrying out our procedures, a line of ants was discovered at a laboratory window. It was not conclusively identified but lab members suspected it was *Monomorium minimum* (little black ant), known to inhabit the Caltech campus area [12] and forage in houses and buildings [13]. One such worker ant was added to the set of samples.

Figure 4: Windowsill in our lab where the ant was captured.

A lab member also added an additional sample consisting of a crumb of sourdough bread (Vons).

4.1 Sequencing methods

A small sample of around 20µL was taken from the anode biofilm of our best-performing microbial fuel cell (currently measuring ≈200mV) from last year's research. This was vortexed before use to prevent separation.

The ant and bread were placed whole in 200µL PCR tubes for further processing.

Lysis buffer was added to samples in a 9:1 buffer-to-sample ratio. An RCA was then performed (Molecular Cloning Laboratories) to amplify DNA present. The product was then purified using magnetic beads (Beckman Coulter), and a PCR was performed with 16S primers[1] (New England Biolabs Q5 master mix) to amplify our target region. The product was purified again by an identical magnetic beads procedure to prepare a library for sequencing on an Illumina MiSeq device for 150 cycles.

4.2 Sequencing analysis methods

Resulting sequences were collapsed to a single forward and reverse read pair per UMI, selecting the longest possible forward and reverse read. Pairs where either read was shorter than 100bp (without UMI) were removed. Reads were then submitted to blastn v2.15.0 [11] on the NCBI 16S rRNA database (version 5). Hits were filtered to at least 95% identity and 100% query coverage and converted to GenBank accession numbers. For each pair, the intersection was taken of the hits on the forward read and the reverse read, including only GenBank accessions that aligned with both ends of the read. The remaining accessions were submitted to Entrez and a list of approximate species identifications in the sample was produced.

5 Results and discussion

[1] AGAGTTTGATYMTGGCTCAG, TTACCGCGGCKGCTGGCAC

5.1 Microbial fuel cell

We saw, in total, 1854 possible GenBank sequences from our data. As a sanity check, we looked to previous work on MFC sequencing [6]–[10] and confirmed in our results the presence of taxa already identified as electrogenic by these earlier authors.

Taxon, in our study	References
Enterococcus	[6]
Salmonella	[6]
Escherichia (besides *E. coli*)	[6]
Aeromonas	[6]
Lactococcus	[6]
Enterobacter	[6], [7]
Citrobacter	[6], [7]
Bacillus	[6], [7], [10]
Staphylococcus	[6], [8]
Klebsiella	[6], [9]
E. coli	[7], [8]
Acinetobacter johsonii	[8]
Dietzia	[8]
Shinella	[8]
Clostridium	[8], [10]
Proteus mirabilis	[9]
Pseudomonas aeruginosa	[9]
Rhodoferax ferrireducens	[9]
Cytophaga	[10]
Flavobacterium	[10]

Table 1: Taxa discovered in our MFCs identified as electrogenic by other authors.

5.2 Ant

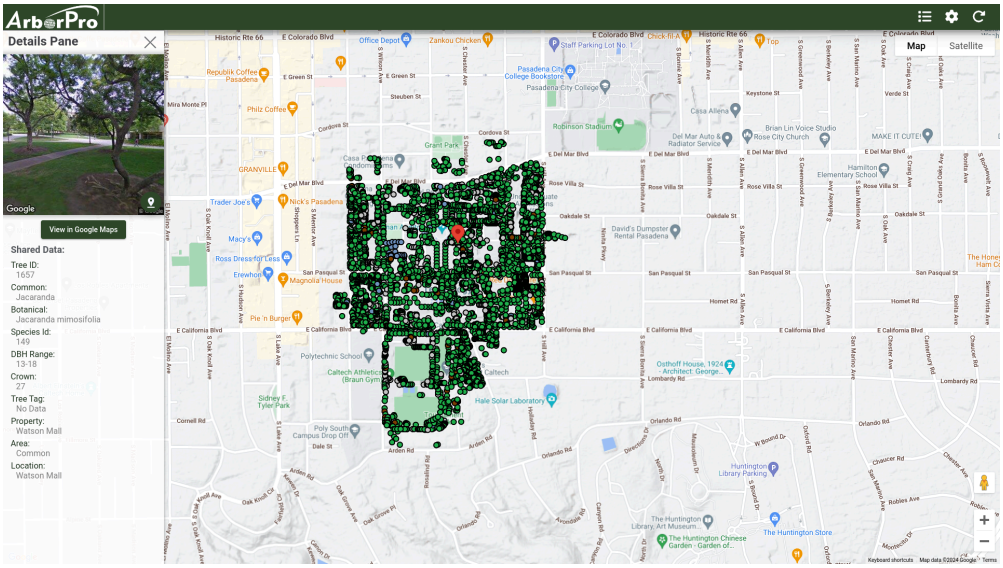

Figure 5: The Caltech Facilities map [14] of trees on campus, with an example tree shown at the northwest corner of Keck Laboratories. We successfully located plant taxa from our ant sample on this map.

We had no expectations whatsoever as to the results of the ant sequencing run. It was conjectured that perhaps DNA of the ant itself would appear. But the results were more startling; many soil bacteria were returned using our method, as would be expected of a primer designed for the 16S gene sequencing an ant almost always in contact with soil. But we also obtained significant (and in most cases, perfect) alignment with plant genomes, particularly those of chloroplasts. We suspect the evolutionary history of chloroplasts as cyanobacteria leads to this genetic alignment across kingdoms. The presence of plant taxa in the results led us to investigate further. After research and consultation we believe pollen to be one of the primary causes. This theory is especially fitting for trees such as the yew pine (see table below) whose pollen is very fine and would readily spread, including onto our ant. Knowing the ant inhabited an urban horticultural environment meant information was likely available on where specimens of our identified plant taxa might be found nearby.

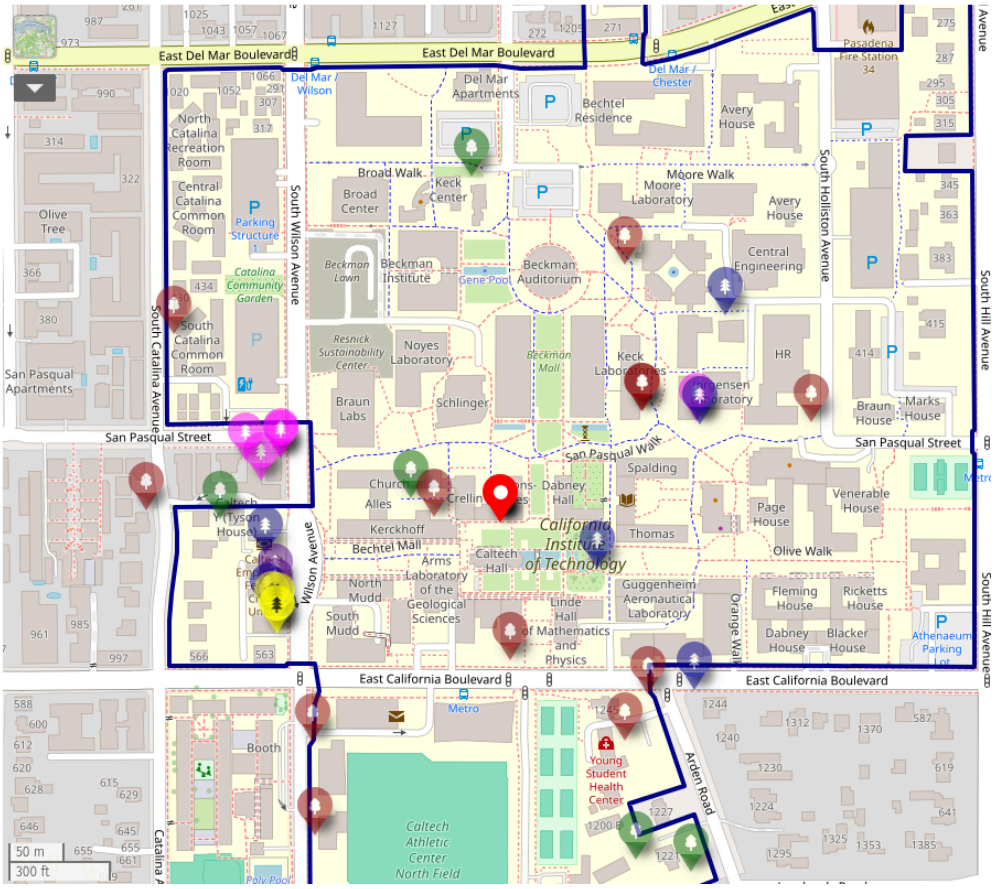

Figure 6: A map of selected specimens of trees found in our sample; our lab, in red, dawn redwood, in maroon, deodar cedar, in blue, ginkgo, in green, yew pine, in pink, Oriental arborvitae, in yellow. Bounds of Caltech are in blue. Data from OpenStreetMap.

Indeed, a map of Caltech campus horticulture [14] is available from Caltech Facilities [15] and we used this to attempt a rough reconstruction of areas on campus where the ant may have frequented. We based our analysis around Gates Annex, where our laboratory is located and where the ant was collected:

Botanical name	**Common name**	**Location on campus[2]**	**Distance from site of ant capture, nearest and furthest specimen**
Cedrus deodara	Deodar cedar	Throop Memorial Garden	100-555m
Ginkgo biloba	Ginkgo	behind Crellin Laboratory (adjacent to Gates Annex)	51-520m
Juniperus chinensis	Hollywood juniper	Fitzhugh House (USGS offices)[3]	207-214m
Metasequoia glyptostroboides	Dawn redwood	Facilities building	296m[4]
Platycladus orientalis	Oriental arborvitae	Caltech Investment Office[5]	217-220m
Podocarpus macrophyllus	Yew pine	Powell-Booth Laboratory, south side[6]	201-567m
Sequoia sempervirens	Coast redwood	Parking lot between Kerckhoff and Church laboratories[7]	54-492m

Table 2: Plant taxa whose DNA were found on our ant, likely *Monomorium minimum* (little black ant), shown against known Caltech tree locations.

[2] closest to Gates Annex

[3] across Wilson Ave from most of campus

[4] only one exists at Caltech to our knowledge

[5] opposite Mudd Laboratory on Wilson Ave

[6] also found at Fitzhugh House across Wilson but we believe the intra-city-block, albeit further, transmission of this DNA is more likely

[7] and many other locations

Figure 7: Trees at Caltech whose species' DNA was found on our ant. Left: A coast redwood (*Sequoia sempervirens*) behind Kerckhoff Laboratories, numbered 2823 [14]. Right: A yew pine (*Podocarpus macrophyllus*) in front of Powell-Booth Laboratory, numbered 2867 [14], and a lab member.

Thus we were able to characterize the spread of plant DNA around Caltech, demonstrating propagation at ranges up to 0.5km. There is a strong likelihood that other species matches in our sequencing data can also be found nearby, but on private property or with otherwise limited information about their presence and location. The list of protected trees for the city of Pasadena also shows significant overlap with other plant species not represented at Caltech itself [16].

We also found various soil bacteria and DNA matching several Apocrita (wasp) species, but we were not able to corroborate or analyze these to a satisfactory degree.

5.3 Bread

No significant alignments were produced for this sample.

6 Data availability

The map of selected trees is available for viewing via OpenStreetMap's uMap system.

7 Acknowledgements

The authors would like to thank:

- Ms. Monica for her leadership and constant support for all we do and keeping us children under control.
- Dr. Matt Thomson for access to the MiSeq machine and other vital supplies.
- Alec Lourenço for his time and many lectures on theory and from his dissertation (on which our sequencing analysis was based), acting as our lab tech, and prepping and running our samples on his own time.
- The Typst typesetting system which was used for this paper.

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