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# Photosynthetic Activity Under Low Light

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## Photosynthetic Activity Under Low Light

By Danijela Lonco

Are bacteria capable of undergoing oxygenic photosynthesis under low-light conditions inside of caves? Inside of an Australian cave, Chlorophyll *f* was recently discovered. This chlorophyll pigment was found to demonstrate the capability for photosynthesis, suggesting that Chlorophyll *f* extends the spectrum of usable light needed for oxygenic photosynthesis (Lars, 2015). Due to the findings in this study, a team went to Carlsbad Caverns to see if there were any bacteria capable of undergoing oxygenic photosynthesis under low light.

To test whether photosynthesis was occurring within the cave environment, samples were collected for DNA sequencing of the ribosomal RNA of the microbial species present. The samples were taken from C11, Goat Cave (GC) and Slaughter Canyon Cave (SCC) at the entrance, twilight locations and dark locations within each cave. My work in this project was to amplify the 16S ribosomal RNA from DNA extracted from the cave samples through PCR. This gene is amplified because the 16S rRNA sequence is used for phylogenetic classifications (Lane, 1985). These genes can be sequenced to identify the taxa an organism belongs to, describe new species, and calculate relatedness between groups. When we began, we thought that the samples were pure cultures, but restriction fragment length polymorphism (RFLP) indicated that there had been no selection for individual species and the DNA represented mixed communities. To determine the structure of these communities, we needed to do Illumina sequencing. For Illumina sequencing, each of the thirty-eight samples was given a specific tagged PCR primer to act as a barcode for identification (Kozich, 2013). PCR reaction contained 50ng template DNA, 25 $\mu$ L Taq Master Mix, 100 $\mu$ M each of forward and reverse primer, and molecular grade water to a volume of 50 $\mu$ L. The primers used in the PCR reaction were 515F and 806R. PCR was started at

95°C for two minutes. This was followed by 30 cycles at 95°C for 45s, 58°C for 45s, and 72°C for 1min 30s. Using 1.5% Pippin Prep gel, the PCR products were electrophoretically size selected and 450bp fragments were isolated. The DNA quantified using a Qbit spectrophotometer. Samples were then sent for Illumina sequencing. The structure of all bacteria present in the samples obtained was then examined using the QIIME software to see if there were any novel photosynthetic bacteria in the cave.

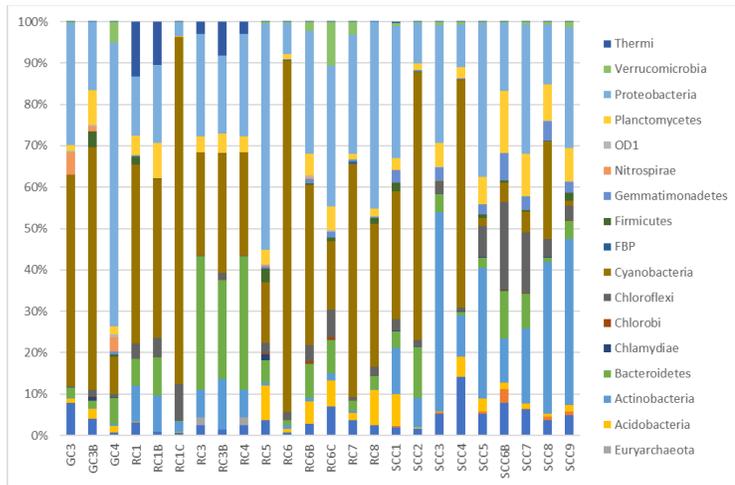


Figure 1: Bacterial phylums present in each sample tested.

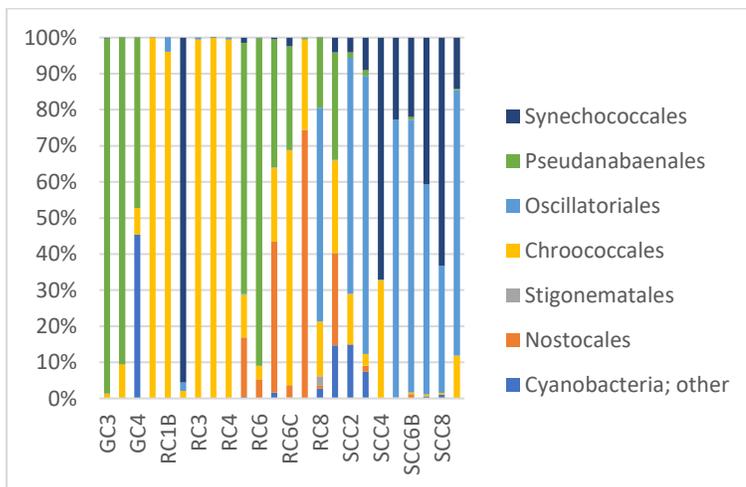


Figure 2: Different types of cyanobacteria found in samples tested.

Out of the thirty-eight samples obtained, only about twenty-four were successfully sequenced using Illumina sequencing. Data from Illumina sequencing showed that each sample was different. Samples obtained from SCC contained fewer percentage of *Cyanobacteria* compared to samples obtained from the other two caves. However, the samples taken from SCC contained higher percentage of *Planctomycetes*, another phylum that utilizes photopigments. Illumina sequencing products shown in Figure 2 were only composed of cyanobacteria. This chart showed that each sample was composed of varied percentages of cyanobacteria classes.

Photosynthetic cyanobacteria have been found inside caves, much further from the entrance than would be anticipated for the amount of light present, pushing the energy limits for potential photosynthesis (Lars, 2015). Light entering the cave refracts off the cave walls, providing energy to these cyanobacteria even in places where no light is visible to the human eye. This was evaluated because there were potentially novel cyanobacterial species within the caves due to photosynthesis occurring in extreme low light and infrared conditions. It took approximately one year for this entire process to be completed. After completion, this data will contribute to the paper, "Light in the dark: Chlorophyll *d* and *f* drive photosynthesis in the twilight zone of caves."

## References

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