Are you lichen it?: The characterization of the Common Greenshield Lichen (*Flavoparmelia caperata*) microbiomes across varying forest microhabitats

Jack Bittner, Rachel Bonham, Jaena Fiscus, Alyssa Jones, Kyle Harris, Matthew Becker

LIBERTY UNIVERSITY

Introduction

The term symbiosis was used for the first time in biological studies while observing crustose lichens by Alberty Bernhard Frank in 1876. Lichens have long been identified as having a symbiotic relationship between algae and fungi. However, recent discoveries in molecular techniques have uncovered that this symbiotic relationship also incorporates bacterial communities. New studies have shown species-specific consistency within the composition of these bacterial communities despite changes in habitats. Additional research has found that lichenization may affect the microbial communities of the lichen substrate. This study will characterize the microbiome across 3 varying habitats within the Candler's Mountain ecosystem in Common Greenshield lichen (Flavoparmelia caperata). Our three sampling areas include a recent clear-cut area, new regrowth of past clear-cut area, and a mature forest area. Samples will also be taken from the surrounding substrate within one inch of the lichen thallus to look for manipulation of the surrounding microbes. Samples will be grown on nutrient agar plates, identified, and compared by morphotype. If the lichens perform symbiosis with particular strains of bacteria and fungi, microbial communities should retain core microbes (members that are present in all samples) despite changes in habitat. Lichen's role as a keystone species makes them an excellent candidate for the bioindication of environmental stress. This research will contribute to the current understanding and development of lichens as monitors for the health of woodland habitats.

Question: What are the morphological differences of the microbiome of *F. Caperata* based on the three locations of old growth, clear cut, and new growth forests.

Working hypothesis: *F. Caperata* has a core microbiome that will remain the same across three distinct microhabitats.

Methods

Field Collection

- Three microhabitats on Candler's Mountain (mature forest, clearcut, and regrowth) will be used for study sites of *F. caperata*
- Lichens will be chosen based on their isolation from other colonies
- The chosen lichen will be photographed and numbered
- Lichen thallus will be swabbed, plated using TSA growth plates, and labeled with GPS coordinates, environment, date and identifying number
- An additional swab will be taken of the substrate, generally measured about a swab's length away. This too is plated and labeled with the above-mentioned identifiers.
- Within each site, 10 samples will be taken of the lichen thallus and substrate
- Samples will then be sealed and allowed to grow in a controlled environment

Lab Work

- Every seven days, growth in the TSA plates will be observed and recorded.
- The culture growths of the lichen and substrate will then be compared to identify and characterize by morphotype

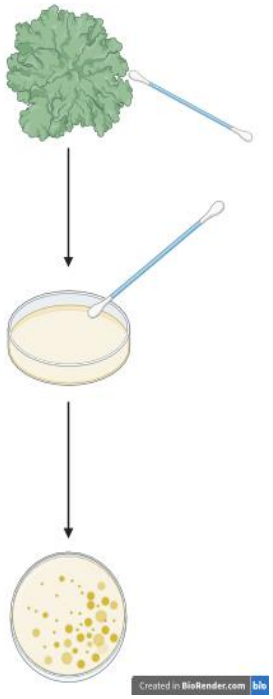

Figure 1. BioRender image created by authors displaying methods and swabbibng technique

Figure 2. Common Greenshield Lichen found in regrowth forest. Image taken by Author

Figure 3. Regrowth forest microhabitat on Candler's Mountain. Image taken by Author

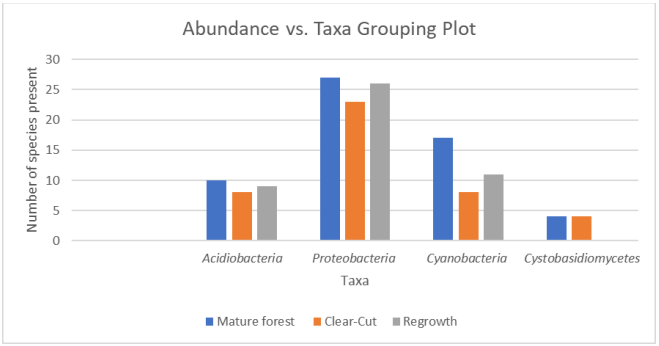

Figure 4. Expected Results of taxa abundance found within microbiome

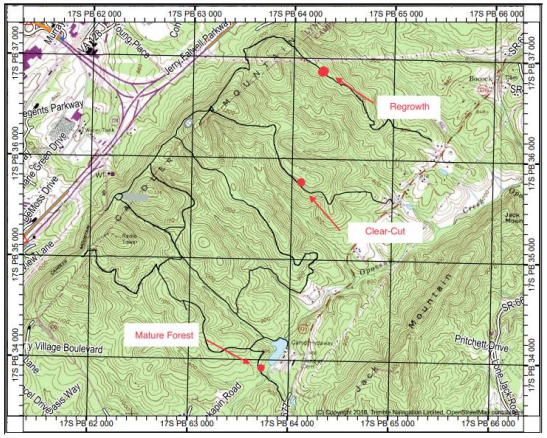

Figure 5. Map of Candler's Mountain study sites. Original map provided by LU Army ROTC and USGS, modified by authors.

Figure 6. Bacterial and fungal growth on TSA plate after preliminary swabbing. Image by Author

Expected Results and Conclusions

Expected Results

- Morphotypes present in all samples will be the same regardless of differences in microhabitat of the study site
- Identification of core microbes across all samples collected
- *Alphaproteobacteria* and *Cyanobacteria* will be found in the core members of the microbiome
- T-tests will show no statistically significant species variance across the three different microhabitats
- Secondary findings will show that lichen samples have a statistically significant species variance from the substrate on which they grow

Expected Conclusions

If species present across all microhabitat samples do not show statistically significant differences, it can be suggested that microhabitat changes do not have a significant effect on the species composition of the microbiome in *F. Caperata.* Additionally, identification of microbes which are present across all samples would further confirm the presence of a core group of microbes within the microbiome. Therefore, it would be suggested that the criteria utilized for the selection of core microbiome members is not based on abiotic environmental factors, but instead specific to the lichen itself. Secondary findings showing that the microbiome of lichen compared to their substrate is significantly different, would further confirm that selection of the microbiome is dependent on characteristics of the lichen and not the microhabitat.

Future Work

1. What are the associations between the lichen microbiomes and the three environments?
2. How are the lichen microbiomes affected by the surrounding substrate?
3. Are there antibacterial substances found within the microbiome of lichen?

References and/or Acknowledgments

Fernández-Brime, S., Muggia, L., Maier, S., Grube, M., & Wedin, M. (2019). Bacterial communities in an optional lichen symbiosis are determined by substrate, not algal photobionts. Oxford University Press (OUP). 10.1093/femsec/fiz012

Hawksworth, D. L., & Grube, M. (2020). Lichens redefined as complex ecosystems. The New phytologist, 227(5), 1281–1283.

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M. C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G., Mayrhofer, H., Johannesson, H., & McCutcheon, J. P. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science, 353(6298), 488–492. http://www.jstor.org/stable/24749976