Investigating potential symbiosis in the gut microbiome of *Cephalotes* ants

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In recent years, various studies have shed light on the importance of the microbial "gut microbiome" to host eukaryotic organisms. Thus, research on unique microbial-host relationships are becoming increasingly important. The ant genus Cephalotes (turtle ant) exhibit one such unique relationship. The most similar relatives to the turtle ants scavenge a variety of food sources. However, the turtle ant's diet exclusively consists of nutrient-poor pollen and nectars. This begs the question: How do turtle ants acquire the essential nutrients lacking in their diet?

Our overarching objective investigates the nutritional symbiotic relationship between the ant and its gut microbiome in geographic space and time. A large component of our research involves culturing the bacterial strains from several Cephalotes species on a variety of agar gel plates. Earlier in the year, Dr. Wertz identified a bacterial strain (dubbed POW232) that was proliferative in the adult ant gut within several different species from different geographic locations. In his plated cultures, POW232 grew exclusively along the border of another colony (CV58), and only did so on agar plates of 5% sheep's blood. This observation allowed Dr. Wertz to conclude that the POW232 strain requires a cofactor or nutrient supplied by the CV58 strain in conjunction with the blood. This combination of cofactors of nutrients would be known as a "symbiosis factor," and its discovery will shed great light onto the mutualistic relationship between the ant and its gut microbiome.

My project this summer focused on determining what this symbiosis factor was. This essentially involved subjecting the POW232 to a variety of growth conditions and qualitatively observing whether POW232 colonies grew. We first confirmed that POW232 grows on blood plates with a variety of other bacterial strains obtained from the ant gut microbiome. This demonstrated that the symbiosis factor must be common in the ant gut, as many bacteria appear to produce it. Further, POW232 grew with a bacterial strain isolated from termite gut microbiome as well as common bacterial strains such as *Streptococcus pyogenes* and *Staphylococcus aureus*. This observation demonstrates that a wide assortment bacteria of various genera support the growth of POW232. However, POW232 would not grow next to all bacterial strains, such as *Streptococcus agalactiae.* This evidence shows us that the symbiosis factor is not universally produced by bacteria. After performing a genomic analysis, we found only one gene present bacteria that supported POW232 growth and not found in bacteria that support POW232 growth: a glycerol-3-phosphate transporter. Presently, this seems unintuitive; there should not be a reason this gene is vital for POW232 growth, and it does not help answer why the blood is necessary for growth. In future experiments, we will be testing what role this transporter may play.

This summer was a great learning experience as I was introduced to a wealth of microbiological concepts and practiced unique laboratory techniques. Our discoveries this summer have simply led to more questions, but I look forward to seeking more answers as I work in Wertz Lab this coming semester. Though it feels like drinking from a fire hydrant, this summer has allowed us to know at least what fire hydrant to drink out of. I look forward to making novel, conclusive discoveries in this fascinating field.