H115 Postmortem Community Dynamics of the Larval Mass Microbiome

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After attending this presentation, attendees will understand the microbial community dynamics involved in carcass decomposition and its potential use as a forensic tool.

This presentation will impact the forensic science community by exploring an unstudied microbial habitat and identifying trends that will aid in estimating Postmortem Interval (PMI).

Recent studies have begun to characterize microbial community dynamics of the skin, soil, gut, and oral cavities during carcass decomposition. One area that has yet to be explored is the larval mass that forms after flies oviposit on carrion. These masses have predictable traits that are of forensic value; for example, the development of these larvae can be used to estimate PMI. The goal is to determine if some of these predictable traits are microbial. The microbiology of this niche will provide novel insight into a common decomposition tool and a regularly encountered phenomenon in death investigation. The current project hypothesizes that the maggot mass, like the carcass as a whole, has a specific decomposer community that changes over time. This project proposes that these changes are linked to both taxonomic abundances and the chemical environment.

Three swine carcasses (*Sus scrofa domesticus*), killed via electrocution, were decomposed in a tropical savanna ecosystem located in Palalo Valley, Oahu, HI, in June 2014. Relative humidity (%) and temperature (°C) were recorded at intervals of one hour. Sampling commenced at 74h postmortem when larval masses were established and at 80h, 98h, 104h, 122h, and 128h postmortem; five swabs were non-destructively sampled at each sampling time. Swabs were immediately placed in sterile tubes and frozen at -20°C. Once the survey was complete, the swabs were subjected to 16S metagenomic sequencing. In addition, chemical readings of the maggot mass were obtained *in situ* using a portable meter with sensors to measure temperature, pH, and oxidation-reduction potential (Eh).

The structure of the larval microbial community shifted whereby three distinct microbial communities were detected, hereinafter referred to as pre-98h, 98h, and post-98h postmortem. The pre-98h community was dominated by phyla Firmicutes (~89%) and Proteobacteria (~19%), both of which were significantly ($p < 0.0001$) more abundant than all other phyla. At 98h postmortem, the abundance of phylum Proteobacteria increased to represent ~77% of the community, while phylum Firmicutes decreased to ~23%. Both phyla remained significantly ($p < 0.0001$) more abundant than other phyla. As PMI progressed beyond 98h, Firmicutes steadily increased and Proteobacteria steadily decreased. Firmicutes dominated (~97%) this final community; it was significantly ($p < 0.0001$) more abundant than all other phyla. This shift was also observed at each taxonomic rank. Further, it was found that Firmicutes and Proteobacteria were significantly ($p < 0.0001$) negatively correlated over time ($R^2=0.999$; $y=-1.02x + 100$).

These shifts can be explained by biotic and abiotic factors involved in the larval life cycle. The pre-98h maggot mass was one of the least alkaline (7.32pH) but most reducing (-295.5mV) environments of this study. This is most likely due to the breakdown of macromolecules into their amino acid constituents and the activity of many lactic acid (*Enterococcus* sp., *Lactobacillus mucosae*) and sulfur/sulfate reducing (*Pseudomonas* spp., *Desulfovibrio* spp.) bacteria lessening the pH and Eh. As larvae grow, more oxygen may diffuse into the maggot mass, decreasing the Eh (-285mV) and increasing the pH (7.45). The drastic shift observed at 98h postmortem could have been achieved in a few different ways. One, this environment had the capability to support aerobic life, which could mean that *Clostridium* spp. (Phylum:Firmicutes) were unable to grow and/or members of family Xanthomonadaceae (Phylum:Proteobacteria) were able to outcompete. Secondly, as the larval mass continued to consume the carcass, adult flies were inoculating the larval mass with Xanthomonadaceae. The final phase was dominated by phylum Firmicutes (~97%) and was more similar to a microbial community indicative of the surrounding environment. For example, *Clostridium* spp. are saprotrophic organisms that are ubiquitous in soils. Overall, a significant ($p < 0.0001$, $F=397$) interaction between time and taxa was observed and it appeared to affect only Firmicutes and Proteobacteria.

Taphonomy, Decomposition, Entomology

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