G71 Using Biolog EcoPlatesTM as an Economical Approach to Determining Postmortem Body Dump Sites Through Microbial Community Level Physiological Profiling

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After attending this presentation attendees will have a better understanding of the role microbial communities play in the rate and ecological dynamics of decomposing remains, and how this information can be used to better understand the timing and postmortem placement of human remains in the environment. Attendees will learn how changes in microbial community level physiological profiles (MCLPPs), during community succession on a body and in the soil beneath, can be utilized to predict the location and duration of decomposing remains.

This novel approach will impact the forensic sciences community by providing a more in-depth understanding of the ecological principles governing microbial community succession. Its cost-effective framework makes it ideal for use in crime scene investigations. On a broader scope this technique will provide insight into the influence of the microbial composition and metabolic products on insect colonization of decomposing remains (i.e., forensic entomology), thus improving the science behind estimates of the period of insect activity (PIA), and hence, that of the postmortem interval (PMI).

Microbial communities are a substantial component of the decompositional ecology and processing of organic material, such as carrion and human remains. Studies in both aquatic and terrestrial systems have shown that microbial communities follow a pattern of succession by metabolizing and modifying resources in a way that makes them usable or undesirable to other organisms, such as insects. While there have been studies describing the succession and diversity of microbial communities involved in carrion decomposition, none have evaluated their potential use for determining the postmortem spatial and temporal placement of decomposing remains in the natural environment. Further, most forensic entomology studies of insect succession suggest that volatile metabolic by-products of this community cue initial blow fly attraction and colonization. Postmortem structural and functional changes in these microbial communities may thus affect the PIA on decomposing remains, having applied importance to estimates of the PMI.

One established and economical method for understanding changes in environmental microbial communities is the use of Biolog EcoPlates™. EcoPlates™ have 31 different carbon sources represented in triplicate on each plate, and were designed for describing entire microbial communities from environmental samples such as soil. The pattern, or signature, of carbon resource utilization by the microbial communities provides MCLPPs. The MCLPPs, calibrated with temperature and genomic sequencing, has the potential to provide ecological data that can predict how long a body has been decomposing, and for how long at a particular location (e.g., on soil).

The objectives of this study were to describe microbial community changes over time (i.e., succession), in a variety of environmental settings and throughout multiple seasons, using Biolog EcoPlates™ in conjunction with pyrosequencing of the microbial genome. MCLPPs from communities on decomposing remains and the soil beneath were

hypothesized to change as a function of succession, and identified stages of succession could be used to determine the stage of decomposition and the spatial and temporal positioning of remains on a rural forest floor. Further, we hypothesized that microbial successional dynamics (community structure rate and sequence of change) would impact initial species-specific blow fly oviposition and colonization. We predicted that MCLPPs could be matched and calibrated with genomic-based methods of describing microbial communities, providing a more economical approach for use in crime scene investigations.

For this study, microbial samples were taken from carrion (swine) (N = 3–9) and the soil underneath (treatment soil) and at two distances lateral (0.25 and 1.0 m) of each carcass (control soil). To understand microbial community structure differences on the carcass, swabs of the buccal, urogential and shoulder skin were evaluated, and all samples were described using Biolog EcoPlates™. This study was done in two seasons and two geographic locations to understand variability and generality of these techniques. In one location, matched samples of each individual sample, or composite sample, were taken and evaluated using the Roche 454 FLX pyrosequencing platform. Each of the samples were analyzed using the bacterial tagged encoded FLX amplicon pyrosequencing (bTEFAP) method to identify patterns of organisms occurring on the decomposing tissue during the longitudinal study and calibrated to MCLPPs from the EcoPlates™.

Preliminary results found substantial change in microbial communities both on the carcass and in the soil

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beneath the carcass, with little change in the control soil communities over time. Variation of MCLPPs among body regions was minimal and could be combined to provide an average body MCLPP signature. During the decay stage of decomposition, MCLPPs were significantly different in soil beneath compared to soil lateral of the body; this supported the hypothesis that MCLPP have the potential to differentiate soil communities where decomposition has been occurring, and possibly predict the time since placement. Further, there was substantial MCLPP variation among inter- replicate body communities, indicating different volatile signatures which could be important to initial blow fly attraction and oviposition location; creating "founder" conditions that could influence subsequent intra- and inter-specific competition, the duration of PIA and, thus, estimates of PMI. Calibration of MCLPPs with metagenomic sequencing is on-going. We will continue to evaluate these communities during multiple seasons and habitats, providing new data important for a better understanding of the ecology of decomposition, and its relevant application to forensic science.

Biolog EcoPlatesTM, Forensic Entomology, Microbial Communities

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