H128 Changes in Bacterial Community Structure and the Estimation of Long-Term Postmortem Interval (PMI)

Michael S. Woolf, MS*, University of Virginia, 6732 Hopton Court, Richmond, VA 23226; Vanessa Sufrin, MS, 11507 Harvestdale Drive, Fredericksburg, VA 22407; Tal Simmons, PhD, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Richmond, VA 23284; and Baneshwar Singh, PhD*, Virginia Commonwealth University, Dept of Forensic Science, 1015 Floyd Avenue, Rm 2015, Richmond, VA 23284

After attending this presentation, attendees will gain a better understanding of the bacterial succession patterns associated with porcine decomposition and their utility in long-term human PMI estimation.

This presentation will impact the forensic science community by providing a new method for long-term PMI estimation.

When human remains are decomposed, investigators ask, "How much time elapsed between the actual death and the discovery of the remains?" Early physiological changes due to autolysis and putrefaction provide some clues and permit reasonable short-term PMI estimations, while insect succession patterns and developmental data allow estimations into the later stages of decomposition. Recent studies on murine, porcine, and human remains have indicated that epinecrotic bacterial succession may provide valuable information regarding elapsed physiological time since death; however, previous studies either had limited replication or were conducted in a laboratory environment. The main goals of this study were to conduct a replicated field experiment on porcine remains for an extended period of time (>1,500 Accumulated Degree Days (ADD), or >60 days), provide information about bacterial changes beginning within a few minutes after death, and develop a method for the estimation of long-term PMI. To accomplish those goals, skin microbial samples were collected from the torsos of the six porcine remains (*Sus scrofa*) every day during the first three days, on alternate days through the second week, and once every week for the remainder of the 60-day period. DNA was extracted using an organic extraction method as described in Zheng et al.[1] Bacterial structure associated with each sample was determined using 16S rDNA MiSeq® sequencing. Sequence data was analyzed using the Mothur pipeline and statistical analyses were performed in R version 3.2.3.[2,3]

Significant changes in bacterial structure were observed across all decomposition stages. Those changes corresponded to changes in the epinecrotic insect communities as well as porcine Total Body Scores (TBS) and Partial Body Scores (PBS). During the initial period of decomposition (from time of death (ADD 0) through the post-bloat stage of decomposition (ADD 149), there was a strong negative linear relationship (R^2=0.7321, y=-2.6411x+8.3845) between bacterial diversity (at 3% genetic distance) and the log 10 of ADD. That initial period of decomposition corresponded with peak adult and larval blow fly activity, as well as with peak Firmicutes and Clostridiaceae abundances; however, across all stages of decomposition, a non-linear relationship was observed (R^2=0.4826, y=1.3968x²-7.298x+12.156). That weak R^2 value is due, in large part, to shifts in resource availability (namely, the skin being swabbed) and insect activity. The diversity scores between replicates began to diverge between ADD 149 and ADD 267, reconverged by ADD 734, and become more disparate thereafter. Shifts in bacterial diversity after ADD 149 corresponded with major shifts in insect activity and with diminishing amounts of skin and increasing bone exposure.

At the phylum level, an inverse relationship exists between relative abundances of Firmicutes, (increases until ADD 149; then decreases through ADD 1703), and Actinobacteria, which decreases until ADD 149 and then

*Presenting Author

increases through ADD 1,703. Likewise, at the family level, an inverse relationship exists between the relative abundances of Clostridiaceae (increases until ADD 209; then decreases through ADD 1,703) and Enterobacteriaceae (increases until ADD 209; then decreases through ADD 1,703).

In conclusion, this study provides evidence that bacterial succession associated with the skin of decomposing remains has tremendous potential for utilization as an indicator for both short-term and long-term PMI estimations.

Reference(s):

1. Zheng L. et al. A survey of bacterial diversity from successive life stages of black soldier fly (Diptera: Stratiomyidae) by using 16S rDNA pyrosequencing. *J. Med. Entomol.* 50, 647-658 (2013).

2. Schloss P.D. et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75, 7537-7541, doi:10.1128/AEM.01541-09AEM.01541-09 [pii] (2009).

3. R: A language and environment for statistical computing. (R Foundation for Statistical Computing, http://www.R-project.org, Vienna, Austria., 2011).

Postmortem Interval, Microbial Ecology, 16S rDNA