



Pathology/Biology Section - 2016

H117 The Utility of Soil Eukaryotes During Human Decomposition and Their Potential Forensic Applications

*Vanessa Sufirin, MS**, 11507 Harvestdale Drive, Fredericksburg, VA 22407; *Tawni L. Crippen, PhD*, Agricultural Research Service, U.S. Dept of Agriculture, College Station, TX 77845; *Jeffery K. Tomberlin, PhD*, TAMU 2475, Dept of Entomology, College Station, TX 77843-2475; *Aaron M. Tarone, PhD*, Department of Entomology, College Station, TX 77843; *Jennifer L. Pechal, PhD*, Michigan State University, 243 Natural Science Bldg, East Lansing, MI 48824; *M. Eric Benbow, PhD*, Michigan State University, Depts of Entomology & Osteopathic Med Specialties, 288 Farm Lane, East Lansing, MI 48824; 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 better understand how the eukaryotic community changes in soil during the human decomposition process and how this information can be used for forensic purposes. In addition, attendees will also be updated on 18S rDNA Ion Torrent™ Sequencing and associated data analysis approaches developed for the determination of eukaryotic community structure in soil associated with human cadavers.

This presentation will impact the forensic science community by providing detailed information on key eukaryotic groups in soil under decomposing human cadavers, whose changes in relative abundance may potentially be modeled for the prediction of time since death.

For more than a century, soil use in criminal investigations has been limited to its chemical and physical properties; however, with the advent of deep sequencing technologies, the microbial communities in the soil associated with human cadavers have the potential to be used in several forensic applications.¹ Many studies have addressed the changing chemical composition of soil beneath a decomposing cadaver; while a few have highlighted the effect this has on the life in the soil, none have yet described the change in the eukaryotic community.¹⁻³ This study investigated how soil eukaryotic communities change when exposed to human decomposition and the potential use of that change in forensic applications.

To achieve this, DNA was extracted using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) DNA extraction method from soil collected beneath and 1m away from six human (three with insect access and three with no insect access) and three porcine remains, every day for five days; the samples collected 1m away from the remains served as the control.⁴ DNA was also extracted from eight of the cadaver sites at a later time, resulting in samples collected between 50 and 415 days since placement. Extracted DNA was amplified and sequenced for variable regions 8 and 9 of the 18S rDNA using Ion Torrent™ semiconductor sequencing following the manufacturer's protocol. Sequences were analyzed using the Mothur pipeline for hierarchical classification.⁵ Resulting data was converted to percent abundance, a square root transformation was applied, then both Analysis of Similarities (ANOSIM) and Analysis of Variance (ANOVA) statistical analyses were performed, where appropriate.

Within 5 days after placement, four out of the nine cadavers had significantly different eukaryotic community structure in the soil beneath the remains when compared to the controls. Differences in percent abundances of eukaryotic taxa were also observed between samples collected beneath the remains within 5 days to those that were collected 50 days to 415 days after placement of the remains. The differences seen between soil samples collected beneath and 1m away from cadavers over the first 5 days cannot be attributed to the decomposition process — too much variation existed within the control soils to comfortably suggest that the changes were a direct result of the decomposition process; however, the fact that eukaryotic community structure looks very different for soil samples collected beneath decomposing bodies 50 days to 415 days after being laid out does show that changes are occurring.

In conclusion, this study provides evidence that the eukaryotic community associated with soil beneath human cadavers can help in the identification of decomposition sites and in estimation of the postmortem interval.



Pathology/Biology Section - 2016

Reference(s):

1. Anderson B., Meyer J., Carter D.O. Dynamics of ninhydrin-reactive nitrogen and pH in gravesoil during the extended postmortem interval. *J. Forensic Sci.* 58, 1348-1352, doi:10.1111/1556-4029.12230 (2013).
2. Benninger L.A., Carter D.O., Forbes S.L. The biochemical alteration of soil beneath a decomposing carcass. *Forensic Sci. Int.* 180, 70-75, doi:10.1016/j.forsciint.2008.07.001 (2008).
3. Carter D.O., Yellowlees D., Tibbett, M. Using Ninhydrin to Detect Gravesoil. *J. Forensic Sci.* 53, 397-400, doi:10.1111/j.1556-4029.2008.00681.x (2008).
4. 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).
5. 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).

Eukaryotes, Forensics, Soil