

H52 A Survey of Bacterial Diversity Associated With Various Life Stages of *Lucilia sericata* and *Phormia regina* Collected From Central Virginia

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After attending this presentation, attendees will better understand bacteria associated with blow flies that colonize human remains. This information will help forensic scientists in improving accuracy associated with the estimation of the Postmortem Interval (PMI) based on entomological and microbial evidence.

This presentation will impact the forensic science community by helping provide a better understanding of carrion resource utilization, blow fly colonization pattern determination, and in improving precision in PMI estimation using entomological and bacterial evidence.

Reliable and valid methods for the PMI determination are frequently sought. Information obtained from the colonization of insects and the related bacterial succession on carrions may be utilized for PMI estimation, but this can lead to erroneous results due to known variations between individuals and species.¹ Apart from the bacterial communities found with human remains, it is important to note what bacteria may be specifically associated with blow flies colonizing these remains immediately after death. Blow flies often associated with carrions in Virginia are *Lucilia sericata* and *Phormia regina*.

This comparative analysis of the various life stages of *Lucilia sericata* and *Phormia regina* was performed as the initial setup of an ongoing study, including various species of blow flies associated with human remains in central Virginia. Although extensive data exists on the biology of blow flies, a well-replicated study on bacteria associated with different life stages of many blow flies is missing. This study characterized bacteria associated with different life stages of *Lucilia sericata* and *Phormia regina* by using 16S recombinant DNA (rDNA) MiSeq[®] sequencing. Five *Lucilia sericata* colonies and four *Phormia regina* colonies were established from female flies collected from Richmond, VA, and nearby areas on beef liver bait. First-generation eggs, third-instar larvae, pupae, adults, and second-generation eggs were collected from each colony for DNA extraction using the organic Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method and dual-index 16S rDNA MiSeq[®] sequencing using the protocol as described by Kozich et al.² Sequences were then analyzed using Mothur version 1.39.4, and statistical analysis was performed using R version 3.4.0.^{3,4}

The majority of bacteria associated with both *Lucilia sericata* and *Phormia regina* belonged to the phyla Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria and are intragenerationally inherited, varying only in their relative abundance. In *Lucilia sericata*, the class Flavobacteria was present at high relative abundance (12.1 %) in pupal samples, whereas its relative abundance was very low (<1%) in all other samples. In *Phormia regina*, the class Actinobacteria was present at high relative abundance (>7%) in the adult samples, where the relative abundance in the remaining samples was very low (<1%). In *Lucilia sericata*, the genus *Vagococcus* was present at high relative abundance in larva, adult, and second-generation egg samples (>12%). In *Phormia regina*, the genus *Lactococcus* was present at high relative abundance in larva and pupa samples (>20%). In both species, egg samples had high relative abundance of the genus *Yersinia*, whereas its relative abundance was very low (<1%) in all other samples.

In conclusion, this ongoing study provides information on the bacterial communities associated with the various life stages of blow flies and their importance in forensics.

Reference(s):

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

Blow Fly, Postmortem Interval, Necrobiome