H13 Microbiome Dynamics in *Lucilia Sericata* (Meigen, 1826) (Diptera: Calliphoridae) Developmental Stages

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Learning Overview: After attending this presentation, attendees will have a detailed understanding of *Lucilia sericata* microbiome diversity and dynamics throughout the developmental stages, as well as new insights on pathogens transmission by this calliphorid species.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees of the complexity of the results, which have both forensic and medical importance, adding novel data on necrophagous insect species' microbiome characterization.

The common green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae), constituted an experimental model in forensic and medical *in vitro* studies worldwide, as one of the first colonizers of decomposed bodies. While microbial dynamics associated to insects could bring complementary information on carcass decay evolution, this study focused on bacterial community characterization from *L. sericata* adult, immature stages and feeding substrate (swine liver), and quantitative transmission of *Salmonella enterica* (Proteobacteria) from adult to teneral specimens using Next Generation Sequencing (NGS) and quantitative Polymerase Chain Reaction (qPCR) techniques.

L. sericata adults were reared under constant laboratory conditions using non-inoculated liver and liver inoculated with low (10^2 CFU/ml) and high (10^4 CFU/ml) *S. enterica* concentrations as feeding substrate. The experiment was performed in triplicate, while insect and liver samples were collected daily.

Bacterial diversity was determined by Illumina® MiSeq® sequencing of amplified 16S ribosomal RNA (rRNA) genes of total DNA extracted from liver tissue and insect specimens. Taxonomic assignment was performed using the SILVA v138 16S rRNA database, while the community analysis and taxa Amplicon Sequence Variants (ASVs) relative abundance were performed in R.

The presence of *S. enterica* in liver tissue inoculated with low and high bacterial contents showed an increase up to $10^{5.3}$x as compared to the initial pathogen concentration. Meanwhile, the quantitative variation of *S. enterica* in insect specimens fed with bacterial-treated liver confirmed the pathogen transmission from adults to larval stages, to pupae, and finally to the teneral specimens. The highest bacterial abundance was reached for the third larvae stage ($10^{5.1}$x), with comparable amounts in the pupae stage. No *S. enterica* could be detected from the non-inoculated liver or from the insect specimens reared in the control jars.

The bacterial communities from liver tissues were dominated by Actinobacteria in the untreated samples, and by Firmicutes in the presence of *S. enterica*, with increased relative abundance in the last experimental days. The highest bacterial diversity was registered during the first experimental days, comprising Lactobacillaceae, Enterobacteriaceae, and Streptococcaceae families, with *Lactobacillus* and *Lactococcus* genera present in all analyzed liver samples. Insect samples presented a greater bacterial diversity during pupae and teneral stages, with Proteobacteria, Actinobacteria, and Bacteroidetes phyla as prevailing taxa, while Firmicutes prevailed the adult and larvae stages communities, with *Lactobacillus*, *Myroides*, *Proteus*, and *Acinetobacter* among the most abundant genera detected.

Overall, these new data on microbial composition and dynamics will strengthen the current knowledge on microbiome characterization from necrophagous insect species, and pathogens transmission by ubiquitous insects.

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