A141 Bacterial Community Succession: Postmortem Interval (PMI) Estimation of Forensic Anthropological Remains

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Learning Overview: After attending this presentation, attendees will understand the need for uniform data collection and sampling protocols regarding the postmortem microbiome for forensic anthropological cases, the composition of the postmortem microbiome of these cases, and whether the genera *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* are useful groups for estimating PMI.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by establishing standard protocols and best-practice guidelines for data collection and sampling the postmortem microbiome and demonstrating how these protocols can improve future research endeavors and collaboration. In addition, this presentation will identify potential trends of bacterial community composition in sample groups not previously studied.

Decomposing remains are an evolving ecosystem experiencing ecological succession as biological species colonize the remains and modify the biochemical characteristics and resources available. Relative abundance and composition of bacterial communities respond to these environmental shifts, resulting in a succession pattern that has been found to correlate temporally with decomposition. Once refined, these patterns may be useful predictors for the PMI. There is a relative paucity of studies, however, that utilize data from actual forensic cases involving human remains. In addition, existing datasets are based on disparate sample groups, taxonomic levels, and sampling locations. In this study, human remains and the corresponding substrates were sampled from two forensic cases from the Mercyhurst University Forensic Scene Recovery Team (M-FSRT) and one forensic case from the Erie County Coroner's Office. The PMIs ranged from 24 hours to more than five years.

Feasible sampling protocols and best-practice guidelines were outlined for studying bacterial community succession on forensic human remains, both at outdoor scene recoveries and in laboratory settings, for future research endeavors. Two locations on the human remains were dry swabbed: around the oral cavity and around the anus. The corresponding underlying skeletal element was swabbed if no soft tissue was present. For laboratory settings, the substrate sampled was the laboratory table. For outdoor scenes, the soil was considered the substrate and sampled in two locations: at 0m (under the center of the remains) and 5m from that center. Four samples were taken for each sample type and location.

Aerobic culture methods were utilized in order to identify a target group for quantitative Polymerase Chain Reaction (qPCR) analysis. The families Lactobacillaceae, Neisseriaceae, and Enterobacteriaceae were cultured from all human remains. Streptococcaceae were only cultured from human remains with a PMI less than 24 hours. Except for Neisseriaceae, the presence of these bacterial families is consistent with significant groups reported in existing literature. *Lactobacillus* was isolated from all cases and was selected for further analysis. Anaerobic genera *Bacteroides* and *Bifidobacterium* were selected based on reports in existing literature.

Primers identified by Hauther et al. were then used to quantify the three aforementioned genera via real-time qPCR.[1] Bacterial DNA was extracted using the Powersoil® DNA Isolation Kit. The concentration of DNA isolated from each sample was determined by Qubit® fluorometric quantification. The sample quadruplet with the highest concentration from each sample was chosen for qPCR. DNA samples were diluted to 1:10 or 1:100 based on performance in preliminary testing of the primers. Differences in presence and relative abundances of all three target genera between sample locations and PMI were observed. Due to the limitations of the sample group, any patterns or observations should not be extrapolated.

Reference(s):

1. Hauther A.H., Cobaugh K.L., Jantz L.M., Sparer T.E., DeBruyn J.M. Estimating Time since Death from Postmortem Human Gut Microbial Communities. *J Forensic Science* 2015; 60(5): 1234-1240.

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