H10 Predicting the Postmortem Submersion Interval (PMSI) Using the Microbiome of Waterlogged Bone

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After attending this presentation, attendees will possess a greater understanding of the use of longitudinal succession of the microbiomes of submerged bone and its use in PMSI estimation.

This presentation will impact the forensic science community by providing information concerning a novel area of research, the use of microbial succession for Postmortem Interval (PMI) and PMSI.

The ability to both identify an individual and estimate the PMSI may depend on DNA obtained from skeletal remains.[1] Currently, PMSI estimation is limited to when insects or other invertebrates have been found, or is based on when the victim was last seen alive, making it primarily applicable in cases of accidental water deaths rather than deposition of homicide victims in water.[2] Because microorganisms are present throughout decomposition, longitudinal succession (including aspects of richness and diversity) of the microbiome of submerged skeletal remains through 16S recombinateDNA (rDNA MiSeq® sequencing was used to provide a reliable method for PMSI estimation. The use of microbes in human decomposition studies began with Vass, when he proposed that the appearance or disappearance of microbes, like insects, could be used to estimate PMI.[3] Although originally abandoned by Vass (as there proved to be too many microorganisms to culture and analyze), recent studies by Benbow et al. and Dickson et al. have demonstrated that characterization of bacterial communities can be a useful tool for estimating PMI and PMSI due to advancements in metagenomic approaches.[2,4] None of these studies, though, have focused on skeletal remains.

In this study, samples from 12 pig (*Sus scrofa*) humeri and 12 pig ribs were divided into a total of 24 humerus and 24 rib samples. Between June and November 2015, cut bones and waterproof dataloggers were submersed in water and left outdoors; water temperature was recorded hourly. A total of six collections of submerged bone were taken at 500 Accumulated Degree Day (ADD) intervals. The collected bone samples were ground into a powder, and the DNA was extracted using ChargeSwitch® gDNA Plant Kit. 16S rDNA variable regions 3 and 4 were amplified with dual-index primer pairs according to Kozich et al.[5] Sequencing utilized Illumina's® MiSeq® 2X300 paired-end sequencing, and analysis was performed via Mothur version 1.36.1.[6] The quality scores were below acceptance; therefore, read one, which covered v3, was used for analysis.

When an Analysis of Molecular Variance (AMOVA) was performed, a significant difference was observed in bacterial structure between rib and humerus samples ($p < 0.0002$), leading to analysis by bone type. Each bone type suggested the following: changes in phylum-level abundance based on greengenes taxonomy classification over time; Principal Coordinate Analysis (PCoA) ordination and UniFrac weighted β-diversity clustering among different time periods; Operational-Taxonomic Unit (OTU) -based indicator taxa for time periods; and, a positive linear increase in Shannon diversity index across time periods for each bone type.

Overall, this study demonstrates that microbial succession may be able to provide a reliable method for PMSI estimation based on the identified indicator taxa and Shannon diversity index.

Reference(s):

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Waterlogged, PMSI, 16S rDNA