A37 Skeletal DNA Preservation and Bone-Associated Microbes: The Implications for DNA Sampling Strategies in Forensic Identification

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After attending this presentation, attendees will better understand microbe-mediated skeletal DNA degradation.

The results of this study provide novel insights into the diverse fungal and bacterial communities capable of colonizing and possibly influencing bone degradation. This presentation will impact the forensic science community by contributing to attendees' current understanding of microbe-mediated skeletal degradation and by informing attendees of current skeletal DNA sampling strategies for forensic identification.

Skeletal DNA degradation occurs at differential rates during human decomposition, and which elements are more susceptible to mechanisms of degradation is currently debated. Some research points to long bones as better reservoirs of high-quality DNA, while others point to small cancellous bones.[1-7] Mundorff and Davoren ranked skeletal elements from three individuals based on their overall DNA yield and ability to ascertain a full forensic profile.[6] This was the first study of its kind that looked at both intra-individual and inter-individual variation in human skeletal DNA yield. That study found that small cancellous bones, on average, yielded greater amounts of better-quality DNA than their larger, denser cortical bone counterparts, a pattern that held true over time in postmortem intervals of up to 21 years.

Yet, the mechanisms behind skeletal degradation and preservation remain largely unknown. Microbial colonization is likely a critical component, since bacteria and fungi readily degrade organic material, including DNA. Therefore, it was hypothesized that microbial intrusion and community composition will be related to human skeletal DNA preservation. While there have been several published reports on microbes associated with soft tissue decomposition, there is little information on the quantity and community composition of microbiota in human bone. The present study seeks to improve understanding of bone-associated microbes and skeletal DNA degradation by looking at bacterial DNA yields and microbial community composition across multiple bone types within and between three skeletons following natural (surface) decomposition.

Human skeletal DNA extracts previously generated and analyzed for Mundorff and Davoren were used to directly compare bone-microbial colonization with DNA quality and quantity.[6] Specifically, extract from 49 bones, from each of the three individuals, were characterized for microbial intrusion using Illumina® MiSeq®. Both 16S recombinant DNA (rRNA) and 18S rRNA gene sequences were obtained for characterization of bacterial and fungal communities co-extracted with human skeletal DNA. Additionally, total bacterial DNA was quantified using quantitative PCR (qPCR), targeting the 16S rRNA gene.

Preliminary results reveal major contributions from the phyla Proteobacteria (20-35%), Actinobacteria (2-11%), Bacteroidetes (2-11%), Firmicutes (1-10%), with additional contributions from Verrucomicrobia, Planctomyetes, Chlamydiae, and Deinococus-Thermus; however, the proportion of these phyla throughout the skeleton do not appear to be uniform and also show varying influences from rarer taxa. Of the fungal communities sequenced,

Ascomycota (40-60%) and Chytridiomycota (15-40%) were highly representative, with lesser contributions by Basidiomycota.

Preliminary qPCR results are highly variable within and between individuals; mean 16S rRNA gene abundances range from 4.10 x 10^8 gene abundances per gram of bone powder in teeth to 1.58 x 10^{10} gene abundances per gram of bone powder in 1st proximal foot phalanges. The bones with the least amount of bacterial DNA were teeth, femora, humeri, ulnae, the 12th ribs, and occipital bones, respectively. Conversely, the highest quantities of bacterial DNA were found in the 1st proximal foot phalanges, 3rd metacarpals, 1st proximal hand phalanges, capitates, and sterna. When considering human DNA yield, the highest human DNA to bacterial DNA ratios were found in teeth, 3rd metatarsals, humeri, mandibles, femora, and the tibiae. Bones with a lower ratio of cortical to trabecular bone appear to be more readily colonized by microorganisms than those with a higher ratio of cortical to trabecular bone.

This is the first study to systematically examine microbial intrusion and microbial community composition throughout the human skeleton.

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Skeletal DNA, Necrobiome, Skeletal Degradation