Using microbial clocks in human cadaver ribs as a postmortem tool

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ABSTRACT

This pilot study investigates the succession of bacterial communities inside the decaying human rib as a potential tool for determining the postmortem interval. Twenty-four ribs were excised from three cadavers at the Applied Anatomical Research Center in Huntsville, TX. One rib was sampled from each cadaver every three weeks over a six-month period, representing over 4000 Accumulated Degree Days. DNA extracted from rib trabeculae was sequenced targeting the 16S rRNA gene region. Results indicate that bacterial communities shift in community membership as time advances, with the biggest trend occurring between the first and last sampling periods. No significant differences in diversity were found between samples. A reliable postmortem interval method cannot be formulated at this time due to the study's small sample size, but the results suggest that there is a trend in bacterial succession that warrants further investigation.

INTRODUCTION

The postmortem interval (PMI) is an important aspect of forensic death investigations, but most PMI tools decline in accuracy with time. While estimations can be determined, they are usually broad in range unless temporal evidence is present. Because microbes drive decomposition, the microbial community on cadaver skin and gravesoil has seen success as a potential clock for determining PMI (1-6). Each of these previous studies shows a shift in microbial succession during the early, wet decay period (1-6). However, as the corpse progresses into the advanced decay stage, the soft tissue dries up and these models becomes less reliable. It has been predicted that the microbial community in human bone will change while it transitions through the later stages of decomposition (7). We suggest that the human rib represents a relatively closed habitat that supports a bacterial community undergoing predictable changes in decomposition. The goal of this metagenomics study is one of discovery and asks three questions: 1.) Are there bacteria inside the decomposing human rib, 2.) If so, does bacterial success occur, and 3.) What changes in succession are we seeing?

RESULTS & DISCUSSION

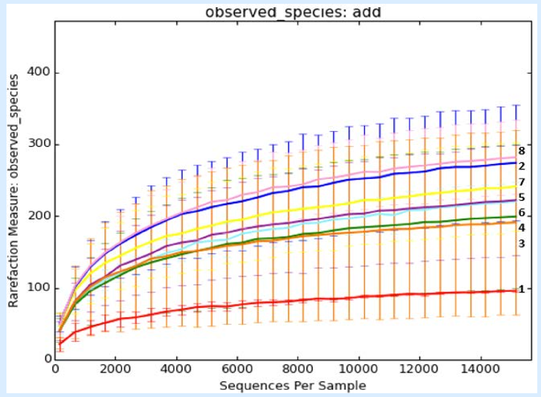
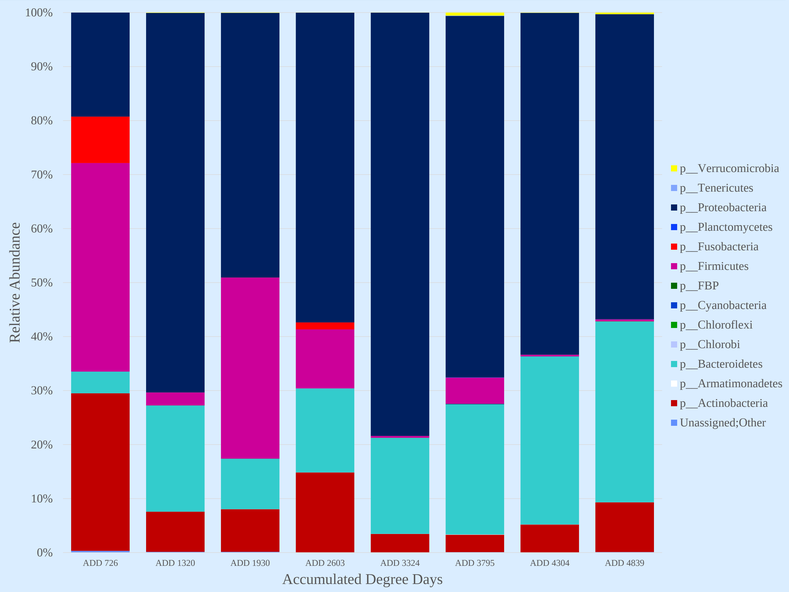


Figure 1: Rarefaction curve of phylogenetic distance at ≥97% similarity, subsampled to 15,167 sequence reads. Each curve represents a sampling time period based on ADD: **1.** 726.9 (red), **2.** 1320.6 (blue), **3.** 1930.2 (orange), **4.** 2603.8 (green), **5.** 3324.9 (purple), **6.** 3795.8 (yellow), **7.** 4304.3 (aqua), and **8.** 4839.3 (pink). Standard deviations are represented by error bars.



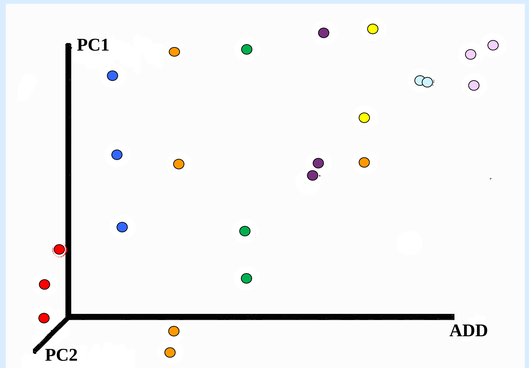


Figure 2: Beta diversity using unweighted UniFrac distances on principal coordinate analysis in Emperor (≥97% similarity). The x-axis is set to accumulated degree days (ADD). Samples are: 726.9 (red), 1320.6 (blue), 1930.2 (orange), 2603.8 (green), 3324.9 (purple), 3795.8 (yellow), 4304.3 (light blue), 4839.3 (pink).

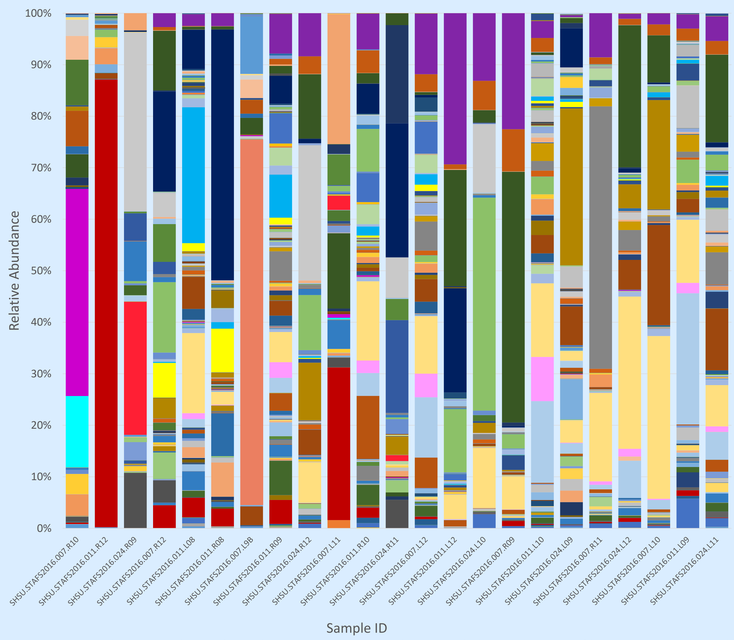


Figure 3: Mean relative abundances for each ADD sampling period at (top) Phyla and (bottom) of lowest taxa represented of each rib sample.

MATERIALS AND METHODS

Human bodies placed at the Applied Anatomical Research Center (AARC) facility in Huntsville, TX, were sampled to determine the predictability of microbial succession in decaying human rib bones. Right and left lower ribs (9-12) were collected approximately every 3 weeks starting at 700 ADD from three bodies placed in Spring 2016 for a total of 24 ribs. A window of bone was excised from the superior margin of each rib, mechanically abraded, pulverized, and washed.

Extraction, Purification and Data analysis

DNA was extracted and purified using the Powersoil® DNA isolation kit from MoBio (Carlsbad, Ca). Bacterial communities were characterized using the 16S rRNA gene regions and sequenced on the MiSeq® Illumina® (San Diego, Ca) platform with the primer set 515F/806R targeting the V4 region. Sequence data was processed and analyzed using QIIME and classified using the Greengenes database. Samples were grouped into "early" (4 groups of 3 ribs) and "late" (4 groups of 3 ribs) ADD based on community dissimilarity using UniFrac unweighted distances.

CONCLUSIONS

Due to the small sample size, significant differences in community composition or diversity between the "early" and "late" sample groups was not found, although a shift in community composition was observed. Community composition changed with the progression of decomposition and nutrient availability, with more differences seen between samples groups (beta diversity) than within sample groups (alpha diversity). The rarefaction data also showed a trend in the increase of richness between the first and last samples, although this trend was not captured statistically. Overall, bacterial succession does occur in decaying human cadaver ribs and may be forensically significant.

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