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Daily thanatomicrobiome changes in soil as an approach of postmortem interval estimation: An ecological perspective

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ABSTRACT

Understanding human decomposition is critical for its use in postmortem interval (PMI) estimation, having a significant impact on forensic investigations. In recognition of the need to establish the scientific basis for PMI estimation, several studies on decomposition have been carried out in the last years. The aims of the present study were: (i) to identify soil microbiota communities involved in human decomposition through high-throughput sequencing (HTS) of DNA sequences from the different bacteria, (ii) to monitor quantitatively and qualitatively the decay of such signature species, and (iii) to describe succesional changes in bacterial populations from the early putrefaction state until skeletonization. Three donated individuals to the University of Tennessee FAC were studied. Soil samples around the body were taken from the placement of the donor until advanced decay/dry remains stage. Bacterial DNA extracts were obtained from the samples, HTS techniques were applied and bioinformatic data analysis was performed. The three cadavers showed similar overall successional changes. At the beginning of the decomposition process the soil microbiome consisted of diverse indigenous soil bacterial communities. As decomposition advanced, *Firmicutes* community abundance increased in the soil during the bloat stage. The growth curve of *Firmicutes* from human remains can be used to estimate time since death during Tennessee summer conditions.

1. Introduction

Decomposition can be defined as the process by which the soft tissues of the body breaks down, resulting in skeletonization [1]. It is a dynamic ecological process dependent upon the environment, climate, insect activity, vertebrate scavenging, microbial activity and intrinsic properties of the individual antemortem [2]. As a body decomposes, five stages can be identified by physical appearance: fresh, bloat, active decay, advanced decay, and skeletonization stages. The fresh stage begins at death and continues until bloating of the carcass is visible. The bloat stage occurs due to microbial metabolic activity that produces gaseous by-products that cause the carrion to inflate. Active decay follows bloating and it becomes obvious when the body begins to rapidly decompose due to insect activity. Advanced decay is characterized by a decrease in entomological activity as the resource is consumed and most of the flesh is liquefied. When all that remains is bones, dry skin, and hair, the carcass is considered to be in putrid dry remains stage of skeletonization [3–5]. As the carcass decomposes, cadaveric material is transferred to the soil when insects and microbes dominate cadaver decomposition, this localized area around the decomposing carcass is known as the cadaver decomposition island (CDI), and its effect is characterized by a localized alteration of soil biology and chemistry [6].

Understanding bacterial decomposition of the human body may be critical in determining time since death or postmortem interval (PMI), and potentially may have a significant impact on forensic investigations. In recognition of the need to establish the

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scientific basis for the time since death (TSD) estimation, several studies on decomposition have been carried out in the last years focusing on the description of the decomposition process itself [7], the exchange between cadaver and environment [6,8], trauma related to taphonomic changes [9] and the significance of entomological evidence [10–12]. Insect succession has been largely used to estimate PMI. The most important implication for PMI estimation is that carrion insect species differ in terms of growth rate, arrival time and position within the order of succession [13]. There are certain concerns for the application of forensic entomology as PMI indicator, like the inaccuracy of the elapse of time between death and egg deposition [14], the lack of insects during particular weather or season [15], or region specific blowfly larval growth curves and insect communities [12]. All of these factors indicate that there are cases and situations where forensic entomology cannot be applied to estimate the PMI. When cessation of life occurs, a depletion of internal oxygen levels takes place, and it allows for autolysis and initiates a widespread cell degradation via anaerobic microorganisms [16] and microbial activity has been known to remain active months after the end of the putrefaction stage due to the increase of carbon level, pH and nutrient concentration in soil [7]. Precise determination of PMI is challenging, and estimative methods used to date are often subjective and limited to the earliest stages of decomposition [17]. Bacteria have an important role in human body decomposition, especially in putrefaction period of decay. Moreno et al. [18] studied the microbial composition of the underlying and adjacent soil in mass graves. The results indicated that measurable changes occurred in the soil bacterial community during the decomposition process. Moreover, the anaerobic bacteria detected were not commonly indigenous to the soil but rather associated with human tissue [18]. Although little is known about the bacterial changes inside and around the cadaver during decomposition, some studies on gastrointestinal tract and respiratory system bacteria have been carried out such as Vass et al., Hyde at al., and Cobaugh et al. [6,18,19,20]. Recent studies have also evaluated the bacterial changes occurred in the soil around the dead body. Facultative anaerobic bacteria, such as *Lactobacillus*, predominate in organ tissues and blood samples (and therefore, they are indicative) of cadavers with short PMI, while obligate anaerobes, such as *Clostridium*, predominate in corpses with longer postmortem interval [21]. According to that, successional changes in thanato-microbiome, or "microbes of death", bacteria can be used to estimate PMI.

Recent advances in the area of soil analysis and the increased recognition of the soil-cadaver interface have marked a shift from traditional studies of the body itself and the aboveground activity of insects and scavengers to the grave soils immediately surrounding it [6,22,23]. Research on soil analysis in a taphonomic context has gained significant momentum in the last 10 years [24]. Moreover, the development of high- throughput sequencing (HTS) platforms has allowed scientists to harness a better taxonomic understanding of the true diversity of microorganisms [5,25].

In this study we used culture-independent HTS of 16S rRNA gene amplicons in order to study in depth the microbiota involved in human body decomposition and its successional changes throughout the time, from the early putrefaction state to the skeletonized state of the body.

2. Methods

2.1. Sample collection

Samples were collected at University of Tennessee Anthropology Research Facilities (ARF) in Knoxville, Tennessee (35°56′28″N, 83°56′25″W). Three donated individuals to the University of Tennessee Forensic Anthropology Center (one male and two females) were studied. Body data collection was performed at the Anthropology Research Facility (ARF) of the University of Tennessee. Data collected consisted of sex, age at death, medical conditions, dental conditions and date of death. Weather parameters, vegetation around the body, and presence of insects were also annotated. Photographs were taken daily and reviewed in order to categorize each cadaver by stage according to Payne's classification [3]. Donor 1 was a white male, of 80 years of age. Donor 2 was a white female of 81 years of age. Donor 3 was an autopsied white female of 27 years of age. None of the donors presented infectious processes related to the cause of death, nor were they embalmed. Soil samples around the body were taken at the different stages of cadaveric decomposition, from the placement of the donated body at the ARF until advanced decay/dry remains stage. Soil samples consisted of approximately 1 g of the top of 0–5 cm of soil next to the cranial area, abdomen and feet of the body. Samples were taken on a daily basis, at the same time of the day. In addition, at each time, a soil sample was collected in the same manner from a control site not exposed to decomposition, located approximately 1 m away from each cadaver. The samples were stored at −20 °C until their analysis.

2.2. DNA extraction and next generation sequencing

DNA was extracted from the collected soil samples using the PowerLyzer PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc., Carlsbad, CA) and PowerLyzer instrument (MOBIO) as a sample homogenizer. All extracts were eluted with 100 μl of RO water. DNA was quantified using a Picogreen fluorescent assay (Invitrogen/Life Technologies Quant-IT assay), 60 μl of each extract was transferred to a 96well plate, and evaporated on a SpeedVac roto-evaporator instrument. Dried DNA plates were stored at 4 °C until their analysis. DNA extracts from the samples were pre-screened for amplificability. DNA extracts were then sent to the Research Technology Support Facility at Michigan State University to perform Bacterial 16S V4 (515F/806R) amplicon library creation, QC and pooling, as well as PE250 base Miseq run of above pool.

2.3. Bioinformatic data analysis

Quality of the 16S rRNA amplicon raw reads were evaluated by using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and sequences with a PHRED score below Q20 or a length of fragment below 150 bp were discarded by using PRINSEQ (http://prinseq.sourceforge.net/). Primers and barcodes were removed and singletons were excluded. In order to avoid biases due to different sequencing depths, all samples were rarefied to 33,000 reads per sample. Sequences were then analyzed using QIIME 1.9.0 software [26]. Operational taxonomic units (OTUs) defined by a 97% of similarity were picked using the UCLUST algorithm. The representative sequences, chosen as the most abundant in each cluster, were submitted to the RDPII classifier [27,28] in order to obtain the taxonomy assignment and the relative abundance of each OTU using the Greengenes 16S rRNA database [29]. Alpha (rarefaction, Good's coverage, Chao1 richness and Shannon diversity indices) and beta diversity measures were evaluated through QIIME. Plotting was carried out in R environment, by using *ggplot2*,*made4* and *factoextra* packages.

3. Results

Air temperatures at the time of the sample collection ranged from 21 °C to 27 °C, with an average value of 24.5 °C. The relative humidity ranged from 71% to 95%, with an average of 81.16%.