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Bacterial community succession analysis by next generation sequencing in Changsha city, China

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ABSTRACT

The estimation of postmortem interval (PMI) is one of the most difficult tasks in forensic practice, especially in putrefied bodies. After death, organisms are decomposed by a variety of enzymes and microorganisms. To investigate the succession of bacterial community in the decomposition process, rat remains were placed outside to decompose under natural conditions in Changsha city, China. Bacterial communities from two regions (buccal cavity and rectum) were sampled when experiment animals were alive, soon after they died and at various time span after death. Bacterial samples were analyzed by high throughput metagenomic sequencing of 16S rRNA gene conducted on an Illumina MiSeq platform. Our data showed that several bacteria genera were potentially useful for estimating the PMI, such as *Streptococcus*, *Ignatzschineria*, *Acinetobacter*, *Aggregatibacter*, *Prevotella* and *Proteus*. There were significant bacterial community structure differences in taxon richness and relative abundance patterns through the decomposition process and across different body sites. As decomposition progressed, a negative linear relationship for taxon richness was found along with a shift from aerobic bacteria to anaerobic bacteria. We first reported the bacterial biodiversity in the decomposition process in Chinese terrestrial scenarios and climatic conditions. Bacteria have a remarkable potential for estimate the PMI and next generation sequencing is a novel method to support the application of bacteria in forensic science.

1. Introduction

Estimation of the postmortem interval (PMI) is one of the most important and difficult practical tasks in daily forensic casework. Most forensic researches with better understanding of how to estimate the PMI entail the study of the physiochemical changes, entomology and environmental factors effects. Compared to other areas of taphonomy, microbial communities exist more continuously in all decomposition stages and may offer a novel and more precise means of estimating the PMI [1]. In recent years, several researches elucidate the microbial community succession that occur as a cadaver decomposes with the potential to be used to establish a "postmortem microbial clock" that can be helpful to assist in PMI estimating [2–4]. However, developing this postmortem microbial clock is not an easy task and we need substantial databases of microbial community sequences in the field of forensic medicine to be accomplished. In our study, we investigate the variation of bacterial community over decomposition process of rat remains in Chinese terrestrial scenarios and climatic conditions.

2. Materials and methods

2.1. Sample selection

The experiments were conducted in Changsha city, Hunan province, central sotuh China (28.12°N, 112.58°E) in July 2013. Six adult female Sprague Dawley rats, each weight 200–220 g, were killed and placed outside with insects allowed access to the carcasses and avoiding direct solar radiation and rain. Bacterial communities of the buccal cavity and rectum were sampled using sterile cotton applicators when experiment animals were just dead, 1 day, 2 days and 3 days after their death.

2.2. DNA extracting and sequencing

Genomic DNA was extracted using MoBio PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA). After PCR reactions (V4 region of the 16S rRNA gene) and purify, high throughput metagenomic sequencing was conducted on an

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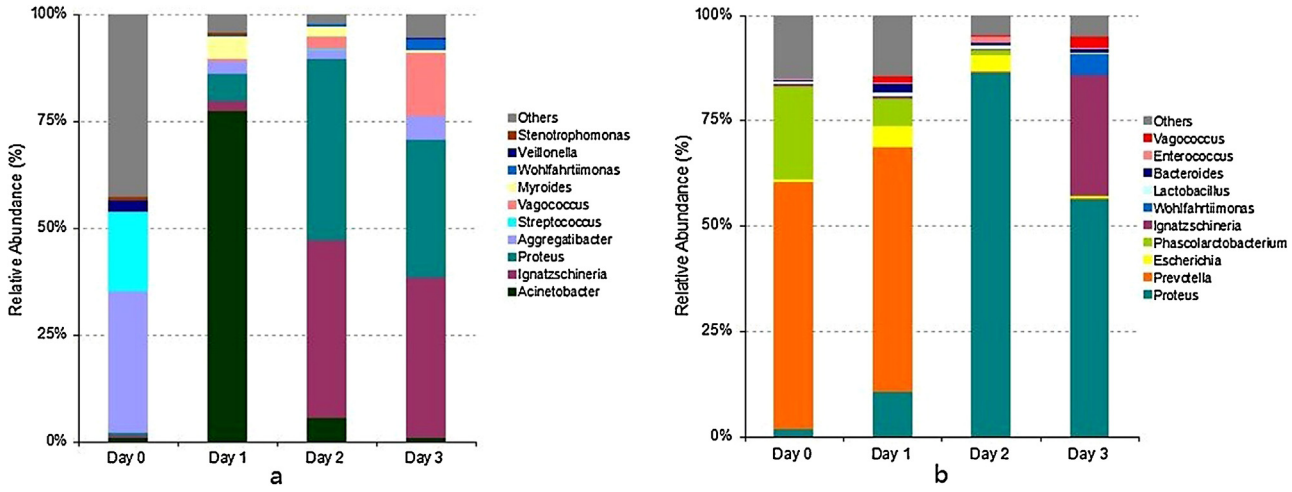

Fig. 1. Bacterial community structure in buccal cavity (a) and rectum (b) at the genus level. Only the top ten genera are listed.

Illumina MiSeq platform and 250 bp paired-end reads were generated.

2.3. Data analysis

Following sequencing, all failed sequence reads, low quality sequence ends, tags and primers were removed. Clustering analysis based on Operational Taxonomic Units (OTUs) of 97% identity and taxonomic classification annotated by each representative sequence were performed. Linear regression between OTU richness over postmortem time was performed with PASW Statistical Software. The differences in community composition among sample regions of samples were tested using analysis of similarities description (ANOSIM) based on the Bray–Curtis dissimilarity distance matrices.

3. Results and discussion

The rat corpses progressed from fresh stage to advanced decay stage during 3 days with the average temperature passed 29.55 °C and humidity revolved around 88.34%. The four sampling time points represented fresh stage, bloat stage, active decay stage and advanced decay stage respectively. A total of 355,278 sequences were obtained after the raw sequences were quality-filtered and the sequences were taxonomically clustered into 1397 OTUs at 97% identity. There was a significant negative linear relationship with OTU richness as decomposition progressed ($y = 526.9 - 125.5x$, $R2 = 0.376$, $P = 0.012$). The bacteria were from 40 phyla, 97 classes, 143 orders, 201 families, and 239 genera. Classification results indicated that 99.21% of all sequences fell into four bacterial phyla, which included Proteobacteria (65.56%), Bacteroidetes (18.01%), Firmicutes (13.84%) and Actinobacteria (1.80%). We also observed a shift from communities dominated by aerobic bacteria to those dominated by anaerobic bacteria. In buccal cavity, *Streptococcus* and *Aggregatibacter* were the dominant genera in fresh stage and sharply decreased 1 day after death; *Acinetobacter* became the most abundant genus in bloat stage and gradually decreased; *Protues* and *Ignatzschineria* were gradually increased to become the dominant genera in decay stage (Fig. 1a). In rectum, *Prevotella* was the most abundant genus in fresh and bloat stages; *Protues* became the most abundant genus in decay stage (Fig. 1b). ANOSIM tests indicated nearly half of top ten abundant genera between samples from buccal cavity and rectum showed significant differences ($P < 0.05$). Our data elucidated the potential use of bacterial succession as postmortem microbial clock once again and contributed to the acquisition of basic scientific knowledge that might have some practical implications for PMI estimation. Furthermore, more animals, sample times, sample regions, different environments and the effect of sarcosaphagous insects need to be considered to establish and correlate the microbes with PMI in the further studies.

Conflict of interest

None.

Acknowledgments

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