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The potential use of fungi community in postmortem interval estimation in China

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ABSTRACT

Body provides a home for diverse commensal microbiota. Microorganisms such as fungi have major roles in this microbial community stability. Postputrefaction fungi have been recorded in association with decomposed mammalian cadavers in disparate regions. The succession and diversity of these fungi are reviewed with a view to their potential as a forensic tool. The researches of mycology would be an interface to forensic investigation and may provide a means to estimate PMI within serious decomposition. To evaluate the use of succession and diversity of fungi species for PMI estimation, we investigate the Internal Transcribed Spacer (ITS) of fungi community on 3 points of time of rat carcasses conducted by Illumina MiSeq platform. Through high-throughput sequencing, several fungi genus such as *Aspergillus*,*Ophiocordyceps* are found in community succession. The community structure of each sample significantly differs in taxon richness and relative abundance patterns within decomposition progress. The succession and diversity of fungi community in corpse decomposition revealed by the indicator of ITS would be a potential forensic tool for PMI estimation.

1. Introduction

Estimation of postmortem interval (PMI) with fair accuracy is a critical step in death investigations. Although many approaches are available to estimate PMI through physical findings and biochemical tests, accurate PMI calculation by these conventional methods is still challenging because it is readily affected by surrounding conditions, including ambient temperature and humidity. In recent years, the research of microorganism have sprung as useful forensic markers for PMI estimation [1,2]. Microorganisms are important in the decay process and influence the presence and concentration of forensic relevance chemicals [3]. It is reported that body provides a residence for diverse commensal microbiota [4,5]. Microorganisms such as fungi have major roles in this microbial community stability and postputrefaction fungi have been recorded in association with decomposed mammalian cadavers in disparate regions [6,7]. In this study, we investigated the succession of fungi community on rat carcasses to establish a microcosm study and evaluated the use of the ITS of fungi for postmortem interval estimation. Temporal changes during carrion decomposition described by high throughput sequencing and members of the postputrefaction fungi communities identified by ITS have been quantified and analysed.

2. Materials and methods

2.1. Sample selection

We used 9 adult female Sprague-Dawley rat carcasses killed by cervical dislocation as models of human decomposition. The experiments were conducted in the summer of Changsha (28.13°N, 112.58°E), China. Fungi communities from rectum of Sprague-Dawley rats were sampled using sterile swabs when experiment animals had just dead (A01), 2 days (A02) and 8 days (A03) after they died. The Sampling time points represent fresh stage, bloat stage and advanced decay stage respectively.

2.2. DNA extracting and sequencing

Genomic DNA was isolated using the MoBio® PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA), mixed in equal concentrations and sequenced the region of ITS via MiSeq (Illumina) system.

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1875-1768/

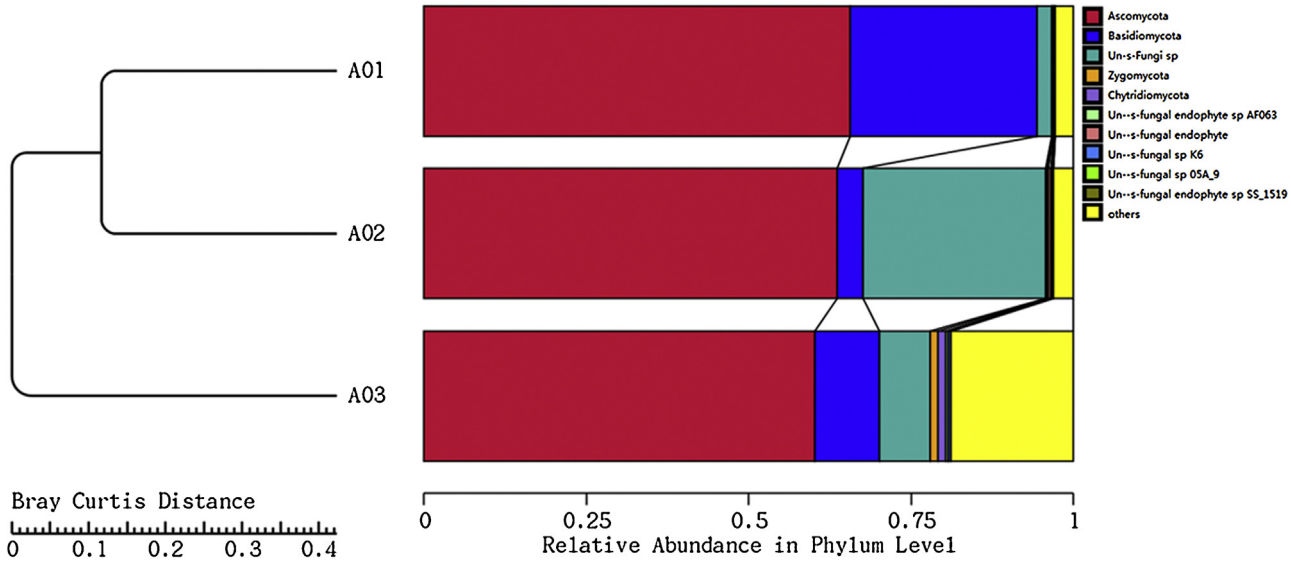

Fig. 1. At phylum level, the histogram of maximum relative abundance of 10 top classifications and the Bray–Curtis distance between each samples.

2.3. Sequencing 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 based on phylum to genus levels were performed. Alpha diversity which used to analyse community diversity within samples was estimated by rarefaction curves, species richness estimators (Chao1 index) and community diversity indices (Shannon index). Beta diversity which used to compare the different community structure between samples was estimated by Bray Curtis distance.

3. Results and discussion

The rat corpses have costed 8 days from fresh stage to advanced decay stage. With the highly decomposition of carcass, rectum of rat could not be distinguished and we stopped sampling and observing.

A total of 130,656 raw sequences (44,202, 44,358 and 42,096 raw sequences for A01, A02 and A03, respectively) were obtained. The reads from A01, A02, A03 were taxonomically clustered into 480, 398 and 285 OTUs at 97% identity. Through species annotation, several fungi phylum such as *Ascomycota, Basidiomycota*, *Zygomycota*, *Chytridiomycota* were found in community and demonstrated marked differences in taxon richness and relative abundance patterns through the decomposition process (Fig. 1). *Ascomycota* was the dominant phylum with the relative abundance of 65.53% and decreased over time. *Basidiomycota* was the secondary most abundant with the relative abundance of 28.86% at fresh stage but decreased obviously in the bloat stage (3.99%) and advanced decay stage (9.86%). The other two phylums of *Zygomycota* and *Chytridiomycota* both increased slowly with the passage of time. With the genus level taxonomic resolution, *Mrakia*, *Aspergillus*, *Amorphotheca*, *Ophiocordyceps* and *Alternaria* were found in community succession within decomposition progress (Fig. 2). The genus of *Mrakia* with the relative abundance of 24.96% in fresh stage decreased obviously at the beginning of bloat stage

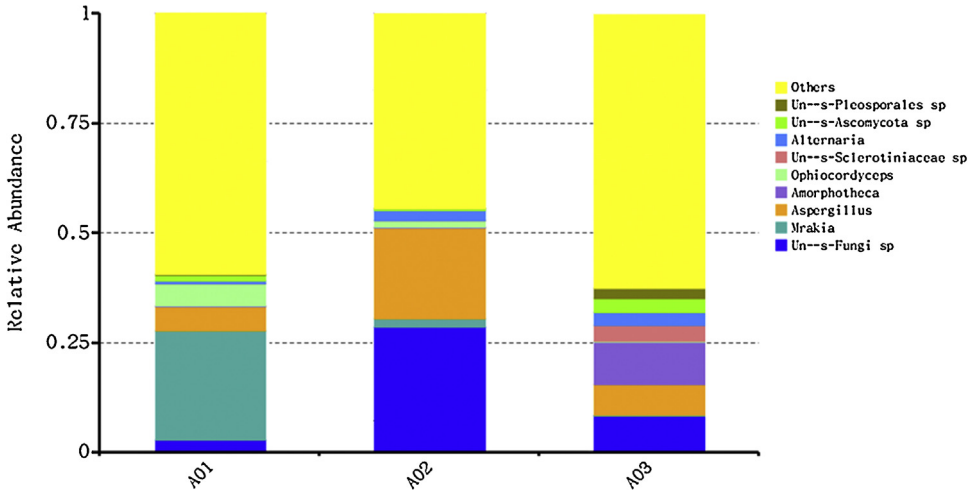

Fig. 2. At genus level, the histogram of maximum relative abundance of major classifications.

(1.75%). *Aspergillus* increased obviously in bloat stage (20.84%) but back to the status of fresh stage (5.5%) in the advanced decay stage (6.98%). The genus of *Amorphotheca* had a higher relative abundance (9.74%) in advanced dacay stage compare to the fresh stage (0.09%) and bloat stage (0.22%). Alpha diversity of each sample indicate that huge species richness in every decomposition stage and there was a positive relationship for overall taxon richness over the course of decomposition. Bray–Curtis distance of A01A02, A02A03, A01A03 was 0.611, 0.856, 0.835, respectively (Fig. 1). Diversity between fungi community structure of each decomposition stage were significantly different.

In our following work, we will augment successive time points for investigating fungi community succession in the decomposition process and establish the correlation between environmental factor and fungi community diversity for PMI estimating purpose.

Conflict of interest

None.

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