H77 A Postmortem Interval (PMI) Estimation Based on Eukaryotic Community Associated With Soil Under Decomposing Porcine Remains

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Learning Overview: After attending this presentation, attendees will understand how eukaryotic community associated with soil under carrion can be utilized for PMI estimation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by helping to increase the accuracy and reliability of current statistical models for PMI estimation and by stressing future research on alternate techniques for PMI estimation with necrobiome sequencing.

Recently, there has been an increased interest in the necrobiome in soils associated with decomposing carcasses. Recent studies have shown bacteria associated with soils under a carcass change significantly both temporally and spatially.[1] However, limited information exists on temporal and spatial changes in eukaryotic community structure associated with soil under decomposing remains. To pursue this technique accurately, more replication in field conditions are needed, as well as a long-term study using a model organism similar to humans. This study was designed to fill in these gaps and expand on previous studies to improve PMI estimation techniques. The main aim of this study was to determine the eukaryotic community structure associated with soil under relatively well-replicated porcine remains for PMI estimation and determination of cadaver decomposition sites. To accomplish this goal, soil samples were collected at 0m (beneath the corpse) and 3m away from porcine remains (N=6). The sample collections started at T0 (Day 0, before porcine remains were placed) and continued daily until Day 3. Samples were then collected on Day 5, then collected weekly until Day 60 (two months, or 1,703 Accumulated Degree Days [ADD]). DNA was extracted from soil samples using DNeasy® PowerSoil Kit following the manufacturer's protocol. The variable region V9 of 18S recombinant DNA (rDNA) was amplified on an Applied Biosystems® Veriti® 96-well thermal cycler utilizing the primers and protocol referred to by the Earth Microbiome Project.[2] The 96-well Polymerase Chain Reaction (PCR) plate included a negative (nuclease free water, Promega® Corporation) control sample. Following amplification, all PCR products were visualized on a 1.8% agarose gel to ensure successful PCR amplification and that the PCR products are of expected size. All PCR products were cleaned using Agencourt® AMPure® XP kit using the manufacturer's protocol. Purified amplicons from each sample were pooled in equimolar concentrations for 18S rDNA dual-index MiSeq® sequencing as described by Kozich et al. on MiSeq® FGx sequencing platform.[3] Sequence data will be analyzed using mothur v1.39.5.[4]

PCR amplification of 18S rDNA yielded two products (one with the expected size and the other with a much smaller size) for many soil samples. The band that had the expected size was gel extracted, cleaned, and used for sequencing purpose. This eukaryotic succession data, or the combination of the eukaryotic and bacterial succession data, will help in reducing errors associated with current statistical models for PMI estimation.

In conclusion, attendees will become aware of the current and emerging techniques used for PMI estimation. The results from this research can be applied to forensic investigations and offer additional detail at crime scenes. Ultimately, this study will aid in expanding the amount of information that can be utilized from evidence in a forensic setting based on necrobiome sequencing.

Reference(s):

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