B92 Bacterial Profiling of Soil Evidentiary Items Using Next Generation Sequencing

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After attending this presentation, attendees will understand how the bacterial composition of a soil sample can be used to link a piece of evidence to a crime scene, victim, or suspect as well as how the passage of time and storage conditions affect the bacterial community present in a soil sample.

This presentation will impact the forensic science community by elucidating the potential for bacterial profiling in tracing soil evidence back to an individual site. Next generation sequencing was used to produce ribosomal RNA (rRNA) 16S libraries that could be grouped at the taxonomic class level based on sequence differences, building a profile of each soil sample. The sequence data were statistically analyzed via a supervised classification technique, assigning evidence samples to a site of origin both through time and under different storage conditions.

Soil has generally been regarded as class evidence in forensic casework. Bacterial profiling has been proposed to help individualize soil samples and aid in tracing evidentiary items to their source location. This study tested the feasibility of connecting evidentiary bacterial profiles to specific locations over time and examined bacterial profile changes under different evidence storage conditions. The research also established whether an evidence sample originating from a specific location and habitat type can be differentiated from nearby locations having the same habitat type. A preliminary study included examination of varied evidence samples to see if bacterial profiles change over time. A shirt, sock, shoes, shovel, and tire were exposed to soil in a deciduous woodlot, then stored at room temperature for six months. Four soil samples were collected from each evidentiary item. Similar changes in bacterial class abundance and membership occurred across all evidence samples.

In the next set of experiments, eight white cotton T-shirts were rubbed in a two-foot by two-foot area of surface soil in a deciduous woodlot on day zero. Four shirts were stored at room temperature (24°C) and four at 4°C. Small (approximately 1cm^2) soil-covered portions of the shirts were collected on day zero and weekly for eight weeks to characterize how soil bacterial profiles changed over time. Surface soil was also collected from the primary deciduous woodlot and eight similar woodlots biweekly to examine whether an evidentiary item can be traced back to a specific location or only classified to a general habitat type, based on statistical comparison of soil bacterial profiles.

DNA extracts were amplified using bar-coded universal 16S rRNA primers and sequenced on an Illumina® MiSeq®. To identify the bacterial classes present, 16S hypervariable regions V3 and V4 were sequenced. Bacterial libraries were filtered to remove ambiguous bases, aligned to a known bacterial sequence, and trimmed to the size necessary for analysis. Sequences were grouped at the taxonomic class level and genetic distances between the samples were calculated using the Bray-Curtis dissimilarity index. A supervised classification technique was used to create a model of the nine deciduous woodlot samples collected over time and to then predict the evidence soil samples' origins.

The soil on the evidence samples was reliably traced back to the primary woodlot immediately following collection and in the weeks following exposure, showing the utility of these methods in individualizing soil evidence. Over time, evidence sample profiles began to exhibit specific class abundance differences from the woodlot where they originated although they never became similar to the other woodlots examined. This measurable change in specific bacterial classes has the potential to act as a biological clock for how long soil has been removed from a habitat. Bacterial class abundance changes on the room-temperature T-shirts mimicked the preliminary evidentiary items study while refrigeration effectively retarded the abundance changes, maintaining similarity to the primary woodlot site. Forensically, this study shows that soil 16S rRNA next generation sequencing shows great potential for tracing soil evidence back to a specific location over time.

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DNA rRNA 16S, Soil Evidence, Bacterial Profiling