B91 The Influence of Distance, Depth, and Time on Forensic DNA Profiling of Soil Bacteria

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After attending this presentation, attendees will understand how the bacterial composition of soil changes over space and time and how this affects its traceability back to a particular location. Attendees will also learn that bacterial profiling of soil using next generation sequencing is a viable tool in criminal investigations by linking evidentiary soil to a crime scene.

This presentation will impact the forensic science community by elucidating how spatial and temporal factors affect the bacterial profiles of diverse soils. Next generation sequencing was employed to develop libraries of bacterial sequences which were compared using multiple statistical techniques to determine their effectiveness in assessing how these factors influence profiles.

Microbial profiling has the potential to individualize a soil sample through the identification and comparison of the tremendous variability that exists in soil bacteria. Next generation sequencing can detect sequence differences at the species or strain level, which is much more sensitive than previous profiling techniques, and allows better classification of the bacteria present in a soil sample. This technique is also becoming more timely and cost effective — important considerations for future implementation of soil bacterial profiling in the criminal justice system.

Temporal effects on bacterial profiles were tested by sampling three habitats: a chemically treated yard, an untreated yard, and a deciduous forest. Surface samples were collected once a day for four days, once a week for two weeks, and once a month for one year. Spatial effects on bacterial profiles were tested by collecting at a center sampling site and 5, 10, 50, and 100 feet from the center in the four cardinal directions. Soil depth was also sampled at the same yard and forest, and a different treated yard, at the surface and directly below at 1, 2, 5, 10, 20, and 60 inches. Sample sets were collected twice, six months apart.

DNAs were extracted using a MO BIO PowerSoil® kit, amplified with bar-coded universal 16S rRNA primers, and sequenced on an Illumina® MiSeq®. Sequence libraries were filtered to remove ambiguous bases, aligned to known bacterial sequences, and trimmed to the region of interest. Statistical pairwise comparisons were performed on all libraries in order to examine whether or not the bacterial profiles were significantly different from one another. Further, variation between libraries was calculated using Bray-Curtis dissimilarity index, which was visualized using a multivariate statistical approach to determine relationships among samples based on their placement in multidimensional space.

Surface soil samples collected from the same location over time associated with each other and also separated from other habitats, both pairwise and in multidimensional space. Spatially, pairwise comparisons and the multivariate statistic showed samples collected near the center point of a yard habitat shared similar bacterial profiles, which became more dissimilar as distance increased. In contrast, the forest samples displayed much more heterogeneity even at short distances. The depth samples showed a similar trend: samples collected at or near the surface (zero to five inches) were similar, but beyond that, bacterial profile differences existed.

The statistical measures largely agreed with each other throughout all comparisons. Multivariate evaluations are valuable as they allow a visual assessment of profile similarities and dissimilarities; however, they do not provide a specific statistical significance value. In contrast, pairwise comparisons can be used to determine significance, but the relationship among all samples is difficult to assess, especially when attempting to determine degrees of difference. Forensically, bacterial profiling of soil via next generation sequencing of 16S rRNA variable regions shows strong potential for tracing soil evidence back to a crime scene over time, bearing in mind that spatial considerations are extremely important.

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