A154 Rhizobial Profiling of Soil for Forensic Applications

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After attending this presentation, attendees will understand the utility of identifying soil via microbial fingerprinting, using a novel quantitative polymerase chain reaction (qPCR) assay designed to determine Rhizobium abundance.

This presentation will impact the forensic community by addressing a novel method for soil identification.

Soil can be of tremendous evidentiary value in forensic investigations, wherein questioned and known samples can either be distinguished from one another or can be shown to have a common origin. Historically, this has been accomplished through physical or chemical examinations, revealing what are functionally class characteristics. More recently, microbial analysis has emerged as a possible way to individualize soils. Within soil there are hundreds or thousands of species of microorganisms, each differing in abundance. These differences can potentially be targeted and assayed, producing a unique microbial "fingerprint" for a given soil sample. However, as with all scientific evidence, microbial profiling of soil must withstand *Daubert* challenges. In this regard, a technique that can generate measurable error rates and is widely accepted in the scientific community would be of great utility in cases where soil evidence plays a large role.

Rhizobia are an ideal group of bacteria for a forensic soil assay, owing to the fact that they are found in virtually all soils, and are well characterized scientifically. However, a present/absent assay, which earlier studies have utilized, may be undesirable because bacterial species that are present at very different levels among soils still test positive, indicating the soils are the same. The goal of this study is to take advantage of well-established frequency variability among soil bacterial species. By developing a quantitative assay that measures the abundance of different rhizobial species known to exhibit high levels of variability in their abundance among soils (largely related to plant species present), one should be able to generate a profile for any soil sample. qPCR has previously been used for microbial profiling, including quantifying rhizobia in experimental settings; however it has not been utilized in a forensic context. The assay presented focuses on the recombination A (recA) gene, as it is one of the most highly conserved bacterial genes, being essential for the DNA repair and maintenance, but still contains hypervariable regions that allow for species identification. These regions were targeted through the utilization of species-specific TaqMan probes. By incorporating different dyes on the probes, multiple species can be detected and directly quantified within a single soil sample. Such quantification allows unique profiles to be generated for different soil types, based on the abundance of the different species assayed. The assay helps decrease run-to-run variability observed in previous studies, lends itself to high-throughput capabilities within a 96-well format, and allows for statistical analysis that will prove vital in translating the assay into a useful forensic application.

Soil Analysis, Bacterial (Rhizobial) Fingerprinting, Quantitative PCR

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