B56 Utility of Soil Microbial DNA Profiling Using Terminal Restriction Fragment Length Polymorphism

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After attending this presentation, attendees will have an understanding of the utility of TRFLP analysis for forensic soil comparisons.

This presentation will impact the forensic community and/or humanity by demonstrating the utility of microbial DNA profiling for the analysis of soil samples, given spatial and temporal variation in these materials.

Soil can be of wide-ranging evidentiary value, in that a soil sample collected from a shoe, tire, clothing, or other material may help associate a victim or suspect with a crime scene. Traditional soil analyses include description of soil type (sand, loam, clay, etc.), particle size and shape, sub- stances in the soil, chemical features such as pH and organic content, and trace elements. While these allow differentiation of soil types to some extent, and may act to exclude a questioned sample as having originated from a site, few objective or statistical analyses exist that can help trace questioned soil back to a specific location. In addition, these methods are very diverse and time consuming, meaning the analyst should have spe- cialized experience in geology and soil analysis for them to be applied to casework.

Beyond the chemical and physical features mentioned above, soils differ in other attributes, including the microorganisms, particularly bac- teria, they contain. These represent a complicated flora that can help define a soil, and are extremely important in determining both local and regional features. Differences in bacterial composition have the potential to help delineate a soil, and thus act as a biological 'signature' that may be useful for forensic purposes. However, the utility of bacterial fingerprinting for soil identification is dependant upon several factors, including the uniqueness of any given soil's bacterial composition, as well as how that uniqueness varies spatially and temporally. If two soil samples from dif- ferent areas have essentially the same bacterial makeup, as might happen if they share a common usage (*e.g.*, a garden), it cannot act as a unique (or even strong) identifier. Likewise, if a single site has a large level of local heterogeneity, soil identification might also be impossible; it is unlikely that the known (exemplar) soil sample will have the exact same origin as the one in question. Finally, the known sample will necessarily be collected after the crime occurred, which may be days, weeks, months after the fact. If the site's bacterial components change during this time, as is quite pos- sible given weather and seasonal variation, the questioned sample may differ greatly from the known.

Though several different methods can be utilized for microbial DNA profiling (*e.g.*, Denaturing and Temperature Gradient Gel Electrophoresis, Single Strand Conformation Polymorphism, Amplified Ribosomal DNA Restriction Analysis), Terminal Restriction Fragment Length Polymorphism (TRFLP) is a useful technique because it can be performed on equipment found in most forensic laboratories. TRFLP is a widely used method for assaying bacterial communities, with its utility spanning from plant root analysis to mammalian intestinal disease. In the current study, TRFLP was used to profile five sites over a one-year period. The method takes advantage of a universal region of the bacterial 16S ribosomal RNA gene that is PCR amplified using a 5' fluorescently labeled primer. The pool of 16S amplicons is digested with the restriction enzyme MspI, the products of which vary in length based on species. These products are sep- arated by capillary electrophoresis, resulting in a multi-fragment TRFLP profile, which can be compared within or among species, resulting in a sim- ilarity index for each.

Soils from five venues: a yard, a woodlot, a sandy woodlot, an agri- cultural field, and a marsh edge, were sampled at the beginning of each month for a one-year period. Each was tested via TRFLP, and the vari- ability among sites assayed. The resulting similarity indexes indicate the uniqueness of each location, and thus the utility of the technique for iden- tifying different soil types. Every third month the same soils were collected, as well as samples from 10 feet in all directions (N, E, S, and W), to assay local heterogeneity, and the effect this had on soil identification accuracy. Finally, through monthly testing over a year's time, temporal variation within each site was measured. Taken together, the results demonstrate the utility of TRFLP for forensic application.

Soil Comparison, DNA and Microbial Profiling, TRFLP

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