B2 Evaluation of Bacterial Community Characterization and Terminal Restriction Fragment Length Polymorphism Analysis for the Forensic Identification of Soil

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The goal of this presentation is to acquaint the audience with new methods for identification of soil samples using terminal restriction fragment length polymorphism (TRFLP) analysis of bacteria. In particular, examination of specific bacterial communities and markers, in lieu of the nonspecific use of the 16S ribosomal RNA (rRNA) gene generally assayed, will be used for soil DNA "fingerprinting" purposes.

This presentation will impact the forensic community by demonstrating the efficacy of bacterial DNA profiling in the analysis of soil samples, given the variation in different microbial communities commonly found in soil, while taking spatial and temporal variation into account.

Soil can be of broad evidentiary value as it is commonly found in many locations that may link a victim or suspect to a crime scene. Soil samples can also help ensure that a body or other evidence was not moved from another location, causing confusion as to the identity of an individual or in reconstructing the crime itself. Soil samples from a shoe, tire, clothing, or any other material may be collected by the crime scene investigator and taken to the laboratory for analysis. Older methods of soil analysis include the physical examination of colors or particle sizes, determination of any other materials in the soil, and examination of chemical features such as pH and organic content. Forensic scientists have also employed the used of light microscopy, density gradient testing, high performance liquid chromatography, scanning electron microscopy, and Fourier transform infrared spectroscopy in an effort to differentiate soil samples. However, these methods can very rarely pinpoint the exact location from which a soil sample originated.

A new method for soil identification involves characterization of the microbial communities found in soils, used to distinguish samples from different locations. There are an almost limitless number of microorganisms, particularly bacteria, present in soil. These differ in species and frequency throughout geographical regions and can potentially be used to help establish the site from which a soil sample originated. Past experiments have examined all bacterial species present in a sample through assay of the ubiquitous 16S rRNA gene. Although results showed that TRFLP was sensitive enough to display community changes, 16S may have been so prominent in soil that it generated a tremendous amount of "background noise", meaning the soil samples could not be properly differentiated. The current research is designed to overcome this by examining DNAs that are found in a more limited group(s) of bacteria, but are still polymorphic enough to "fingerprint" soil samples.

Other factors such as temporal and spatial variability may also play a large role in bacterial community profiling. If, for example, two samples come from similar habitats or soil types, the resulting fingerprints may be too similar to definitively differentiate them. In a like manner, if a geographic region contains a large amount of variability among nearby locations, it may reduce the possibility of obtaining informative results even if a good soil fingerprint can be obtained, as a

known soil sample is unlikely to be obtained from the exact same spot as the questioned material. Finally, the makeup of the soil may change temporally, making comparisons difficult or impossible. This research takes these factors into account while examining the usefulness of bacterial community analysis in a forensic setting.

DNA Profiling, Soil, Bacterial TRFLP Analysis

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