TECHNICAL NOTE

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Forensic Comparison of Soils by Bacterial Community DNA Profiling

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ABSTRACT: This preliminary investigation has shown that a soil microbial community DNA profile can be obtained from the small sample of soil recovered from the sole of a shoe, and from soil stains on clothing. We have also shown that these profiles are representative of the site of collection and therefore could potentially be used as associative evidence to prove a link between suspects and crime scenes. Soil community profiles were obtained using the T-RFLP fingerprinting method that uses fluorescent primer technology and semi-automated analysis techniques similar to those used in human DNA profiling in forensic laboratories.

KEYWORDS: forensic science, soil, DNA, bacteria, fingerprinting, forensic testing

Soils are complex systems consisting of finely divided crystalline and amorphous minerals, inert and decomposing organic matter, animals, plants, pollens, microbial residues in various stages of decay, and a living, metabolizing, microbiota (bacteria, fungi, algae, yeast). This mixture is impacted by abiotic factors such as topography, climate, parent materials, duration of soil formation; and biotic factors such as macro and micro flora, and human activity (1,2). In the forensic context, soil analysis can play an important role in a criminal investigation as trace evidence that can link a suspect to a crime scene.

Forensic soil characterization has, for the most part, relied on physical properties such as color comparison, mineral examinations, or density gradient distribution (1,2). However, these types of techniques require a larger sample size than is normally encountered in actual case situations (e.g., soil from a shoe), and consequently, most of the techniques applied by soil scientists are not easily applicable to the forensic purpose (1,2). Soil analysis is seldom carried out in routine forensic examination, principally because the range of skills needed and the years of experience for the investigator are seldom available. In New Zealand, comparison of pollen distributions in soils are often carried out to characterize soil samples (3,4), principally because the range of skills and experience are available.

Analysis of soil microorganisms has to date been ignored by the forensic scientific community. This is mainly due to the limitations of traditional culturing techniques, which allow only a small subset of organisms to be isolated and characterized (5–7). However, the rapid growth of molecular biology has resulted in techniques that can circumvent the requirement to isolate and culture microorganisms as a prerequisite to identification. Soil microbial diversity is now routinely characterized using simple molecular techniques based on amplified ribosomal RNA (8–12). To date, molecular analysis of microbial diversity and community composition has been used to analyze microbial populations in many diverse environments (5,12,13). The potential exists for such technologies to be used for forensic purposes. This paper describes the first investigation of microbial community analysis for the forensic characterization of soils.

Methods

Scenario 1—Footwear Impression

At Site A, a shoe print was made and adhering soil was recovered from the tread of the shoe outsole (A1). Soil was also collected from the shoe print (A2). Eight months later the exact location of the shoe print was revisited and a further soil sample was taken (A3). Reference soils (approximately 50 g wet weight), were collected from five different locations for analysis (Table 1). Soils were characterized by the "Feel" method (14) and then homogenized by sieving to <2 mm and stored at 5°C until use. Sample identity was not made known to the analyst.

Scenario 2—Soil Stained Clothing

An imprint was made by kneeling in the soil, wearing a clean pair of jeans, at another site. Soil was sampled from the impressions made by each knee (left and right). The jeans were taken back to the laboratory and areas of soil staining removed for analysis. The left knee soil stain was extracted with water; the right knee stain was extracted with water and Tris-EDTA (TE) buffer, pH 8.0.

DNA Extraction

Microbial nucleic acid suitable for Polymerase Chain Reaction (PCR) analysis was extracted from 500 mg of soil using the

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FastDNA Kit for Soil™ (Bio101, CA). Soil bacterial profiles were generated using Terminal Restriction-Fragment Length Polymorphism (T-RFLP) analysis (11,13). Briefly, primers were chosen to amplify a selected region of the 16S rRNA gene of the bacterial soil community DNA (15,13), and the amplified product was approximately 1300 bp. Each primer was labelled at its 5' end with a phosphoramidite dye (Applied Biosystems, Australia). PCR products were digested with restriction enzymes (13), and the fluorescently labelled terminal restriction fragments were separated using the ABI 310 genetic analyzer (Applied Biosystems, Australia).

The data was analyzed using the GeneScan 3.1 software program (Applied Biosystems, Australia). The output of the analysis consists of an electropherogram (Fig. 1), which shows the terminal restriction fragments of the digested products. Variations in the number and size (±1 bp) of the peaks (i.e., peak presence or absence) for each profile were compared and a Sorenson's similarity index calculated (16,12):

$$Cs = 2N_{AB}/(N_A + N_B)$$

Where

Cs = Sorenson's similarity index
N_{AB} = number of matching peaks (±1 bp)
N_A = total number of peaks in Soil A
N_B = total number of peaks in Soil B

DNA fragments (peaks) between 75 and 490 bp with peak heights over 150 fluorescence units were included in the analysis. An arbitrary cut off point of 150 fluorescence units was chosen. This will be the subject of further validation work.

TABLE 1—*Soil characteristics of forensic and reference samples from Scenario 1—footwear impression.*

Site	Location	Soil Type
A–forensic sample, soil recovered from outsole of shoe (A1), soil recovered from shoe print (A2), soil collected from shoe print 8 months later (A3)	Back yard, Maungaraki, Lower Hutt, North Island, New Zealand	Clay loam
B–reference sample	Back yard, Maungaraki, Lower Hutt, North Island, New Zealand	Silt loam
C–reference sample	Back yard, Taita, Lower Hutt, North Island, New Zealand	Silty clay
D–reference sample	Park, Kapiti Coast, North Island, New Zealand	Fine sandy loam
E–reference sample	Back yard, Gracefield, Lower Hutt, North Island, New Zealand	Clay

Results and Discussion

Scenario 1—Footwear Impression

The microbial community profiles for soil from the shoe print and soil collected from the shoe itself produced a very high (>0.9)

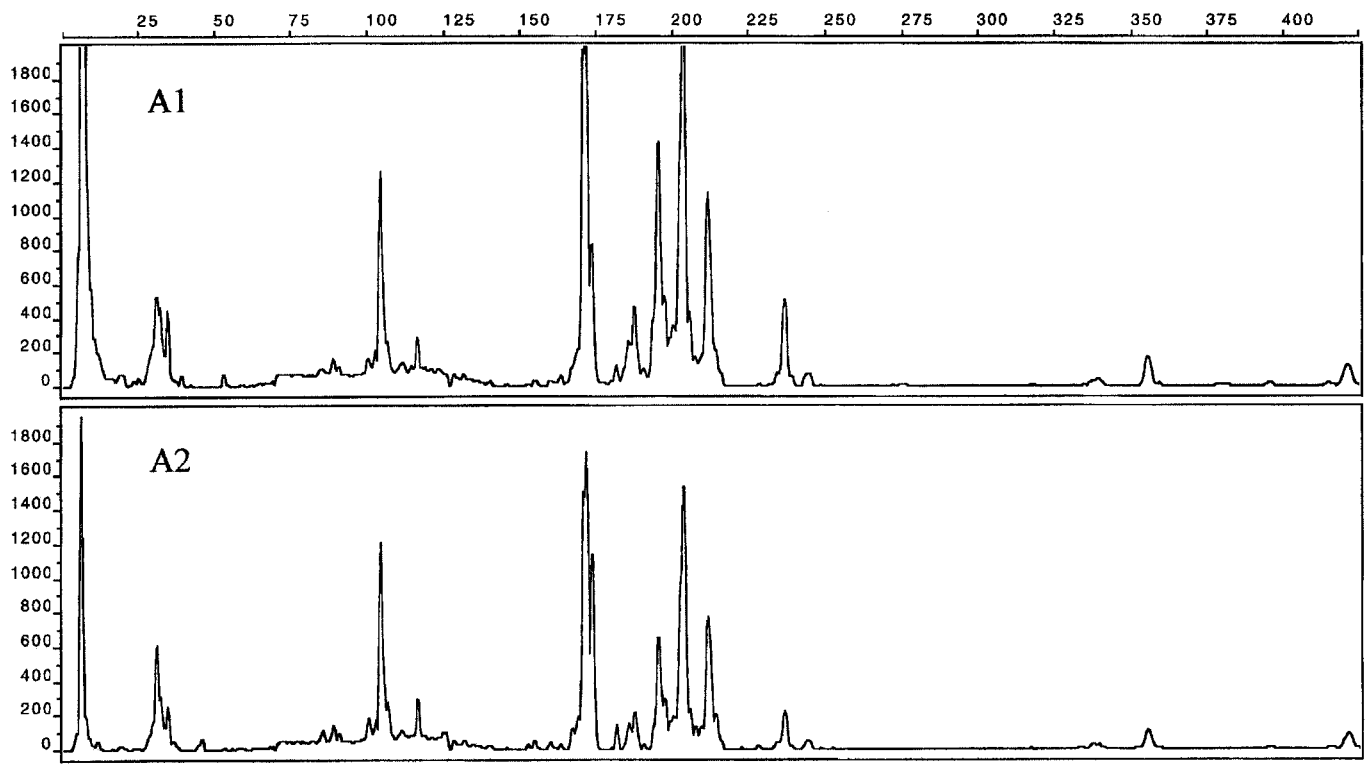

FIG 1—*Electropherograms of the soil microbial community profiles from Scenario 1—footwear impression; soil collected from shoe (A1) and soil collected from shoe print in soil (A2). Electropherograms have been aligned by base pairs (fragment size in base pairs is shown on the X axis), peak heights are shown as fluorescent units detected (Y axis). Sorenson's similarity index = 0.91, peaks called are those between 75 bps and 490 bps and peak heights over 150 fluorescence units as stated in the methods section.*

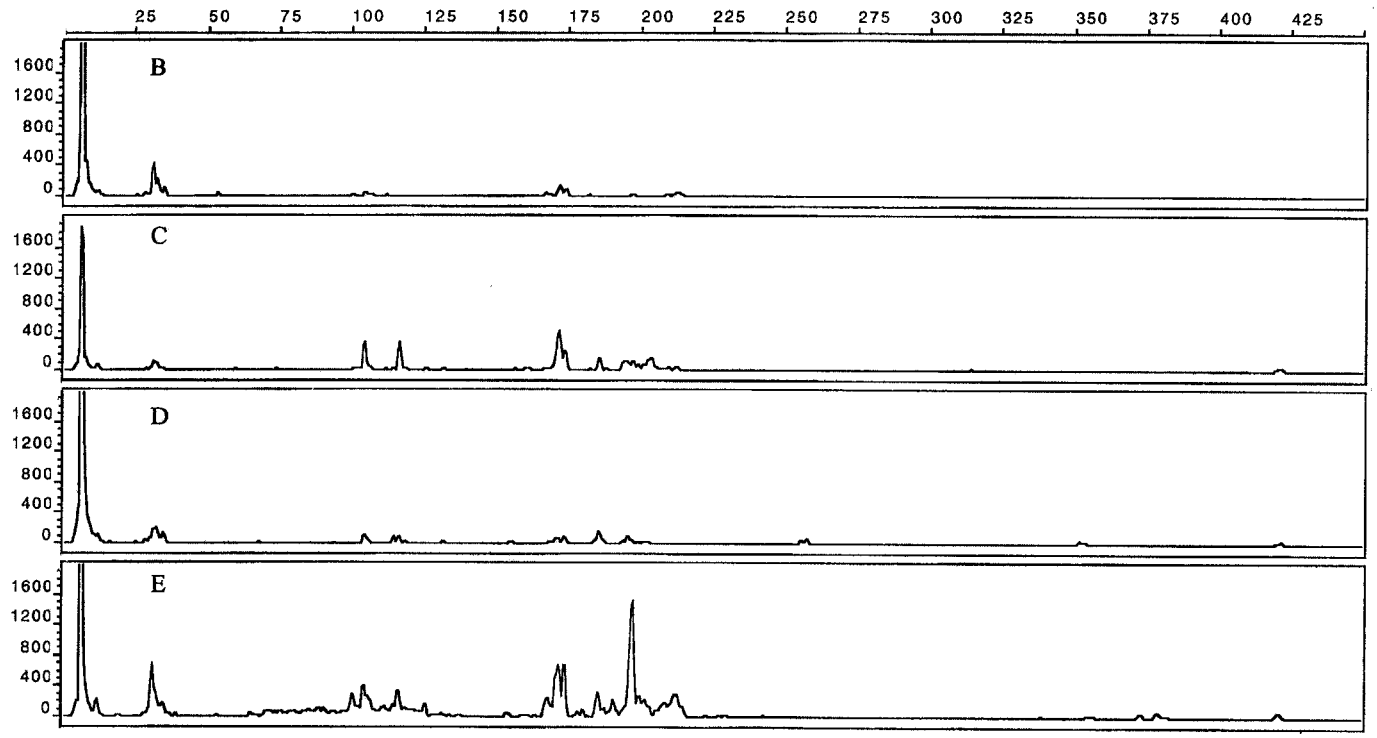

FIG. 2—*Electropherograms of the soil microbial community profiles from reference soil samples in Scenario 1—Footwear impression. Electropherograms have been aligned by base pairs (fragment size in base pairs is shown on the X axis), peak heights are shown as fluorescent units detected (Y axis). Peaks called are those between 75 bps and 490 bps and peak heights over 150 fluorescence units as stated in the methods section.*

TABLE 2—*Similarity index for forensic and reference soil samples in Scenario 1—footwear impression.*

Site	A1	A2	A3	B	C	D	E
A1							
A2	0.91						
A3	0.62	0.70					
B	0.54	0.53	0.56				
C	0.59	0.67	0.61	0.63			
D	0.56	0.64	0.57	0.59	0.73		
E	0.48	0.64	0.64	0.57	0.62	0.50	

similarity index (Fig. 1). In contrast, there were major differences between the profiles of the reference soils and those of the crime scene and the suspect's shoe, with a similarity index of <0.6 (Fig. 2, Table 2).

When the soil sample collected eight months later (A3) was compared with the microbial community DNA profile of soil collected at the time of original sampling (A2), differences were observed. Although, overall, there was a degree of similarity between the profiles and a similarity index of 0.70 was obtained. This was far lower than the similarity index of 0.91 found between the original shoe print (A2) and soil collected from shoe (A1). Differences in profiles with time are not an unexpected result as seasonal fluctuations in parameters such as rainfall and temperature, may impact on the microbial community causing population shifts (12,17,18). Fisher and Triplett (1999) found similar differences in microbial community profiles when they sampled a freshwater lake in June and three months later in September, a Sorenson's similarity index of 0.74 was found.

Scenario 2—Soil Stained Clothing

The microbial community profiles for the soil from the left knee impression in the soil, and the soil water extracted from the left knee of the jeans were very similar, with a Sorenson's index of 0.82. The similarity index of the right knee soil impression and the water extract and TE buffer extract were also very high (0.78 and 1.00 respectively).

Although this study represents preliminary work only, we have been able to show that a soil microbial DNA profile can be obtained from the small sample of soil recovered from the sole of a shoe i.e., with sample sizes likely to be encountered in forensic casework. Profile comparisons using a simple similarity index indicated that soil samples from the same location had a greater degree of similarity than soils from a different location. Soil microbial community profiles can also be obtained from soil stains on clothing, in this instance a pair of denim jeans. We have shown that this profile is representative of the site of collection.

The results obtained using this method should be interpreted cautiously. Because the molecular technique relies upon total community DNA extraction and PCR amplification, it is subject to biases introduced by these procedures. For example, bias related to DNA extraction (19), or subsequently during PCR amplification: preferential denaturation due to overall low GC (guanine and cytosine) content; preferential annealing to particular primer pairs; differential accessibility of rRNA genes; and re-annealing kinetics when product concentrations exceed threshold values (20–22). In addition, specific biases associated with amplification of the 16S rRNA region, such as possible preferential amplification of shorter templates, and correlations between amplification probabilities and gene copy numbers within genomes, have not been thoroughly investigated. However, the results show that microbial community

analysis can be used to estimate composition of natural samples (such as soil). The resulting electropherograms can be readily compared between sites, and the degree of similarity between soils can be estimated. Before this system can be routinely applied to forensic casework the methodological parameters that can significantly influence variability in profiles will need to be determined. For example, DNA isolation, PCR conditions, and PCR product digestion will need to be optimized. In addition, an extensive validation study will be needed to determine variation within and between soil sites (in this study a similarity index of 0.73 was found between soils collected from different locations, Table 2, Soils C and D), with distance, depth and time, and the effects of sample drying, sample ageing, and contamination.

Sorenson's similarity index used in this paper is not an optimum indicator of sample differences or similarities, because in most cases, the main differences between profiles may not only be the presence or absence of peaks, but in addition, the relative intensity (peak heights). More sophisticated approaches to the statistical interpretation of the significance of matching soil DNA profiles are being sought.

We envisage that the main forensic application of this technique will be in providing associative evidence, i.e., assisting to prove or disprove a link between people and objects with places or with other people. For example, unknown soil on a suspect's clothing or shoes could be compared with a known control soil sample from the crime scene. Other investigative applications may be possible, such as determining where a vehicle has been using analysis of soil adhering to tyres or wheel arches. This method will not replace other soil comparison methods (e.g., pollen analysis, color comparison, mineral examinations, and density gradient distribution), rather it can provide a rapid screening method that can be used routinely in forensic laboratories that does not require a high level of skill or years of experience.

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