H55 Exogenous Factors Affecting Bacterial Profiling of Soil on Clothing Via Next Generation Sequencing

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After attending this presentation, attendees will understand that bacterial profiles derived from soil and recovered from clothing are a viable source of trace evidence despite exogenous variables affecting the profiles.

This presentation will impact the forensic science community by illustrating that factors affecting soil bacterial profiles do not entirely disrupt the profile, which gives soil identification improved utility in forensic analysis.

Soil is potentially a valuable form of trace evidence, helping associate an object on which it is found with a crime scene. Historically, soil has been analyzed via visual and chemical class characteristics. Today, molecular tools have expanded the scope of forensic soil analysis to include microorganisms (e.g., bacteria, fungi). Most recently, Next Generation Sequencing (NGS) allows the millions of DNA sequence reads to be used to identify soil microbes and determine a microbial profile that distinguishes soil samples.[1] To be useful in forensic applications, stability of these profiles is key, as factors that change an evidential profile could nullify an association with the soil's place of origin.

Soil on clothing has been shown to be a viable source of evidence through bacterial profiling of the 16S ribosomal RNA (rRNA) gene; however, such studies did not involve a person wearing the item.[2] It is possible that the human skin microbiome or body fluids (e.g., blood) might alter a soil bacterial profile on clothing, either by augmenting the bacteria present or through selection for or against certain bacteria when exposed to a new growth environment. Understanding the effect of such variables is key for the use of soil bacterial profiling as a forensic resource.

In this study, 12 participants wore new T-shirts for 24 hours (in accordance with Institutional Review Board (IRB) approval), and cuttings were taken from the back of each worn T-shirt. The T-shirts were then exposed to soil from four different habitats and stored in paper bags. At the same time, pure soil samples from the habitats were aged in weigh boats. Cuttings/samples were taken from each at day 0 and monthly for six months. In a separate experiment, soil from a habitat was mixed with fresh pig blood in three different ratios (1:10, 1:1, and 10:1 soil:blood) and placed on clean T-shirts. Three T-shirt replicates were either stored in plastic bags (wet) or allowed to air dry and stored in paper bags. Soil/blood cuttings were taken from each T-shirt at day 0, week 1, month 1, and month 2.

DNA was isolated from all soil samples using a MO BIO PowerSoil® kit. A 500bp stretch of the 16S rRNA gene containing variable regions 3 and 4 was amplified using universal, barcoded primers. Amplicons were purified and sequenced on an Illumina® MiSeq®. Bacterial profiles from the T-shirts were visualized using abundance charts and associated via non-metric multidimensional scaling. A random forest algorithm was used to objectively assign the soil evidence to habitats, and scores were generated that reflect the confidence of that classification.

Bacterial profiles were obtained from each worn T-shirt and all soiled cuttings. In all instances, the T-shirt soil profiles correctly classified with the habitat of origin, and there was no detectable influence from human microbiomes. For T-shirts exposed to soil and blood mixtures, the effect of blood on the soil bacterial profiles differed depending on storage type, time since exposure, and the soil/blood ratio. All treatments resulted in very similar bacterial profiles on day 0, which was maintained in the dry T-shirts and those with little blood (10:1); however, the wet T-shirts at 1:1 and 1:10 ratios differed markedly from the other samples after week 1 and through month 2. Notably, *Bacilli* and *Gammaproteobacteria* increased substantially in bloody wet T-shirts, bacterial classes that include species that are known blood pathogens.

The results demonstrate that soil on clothing can accurately and reliably associate with soil from a crime scene. The soil dominates the bacterial profile of samples exposed to the human skin microbiome or to small amounts of blood. Even when blood-saturated, dry storage or immediate sampling allows the soil on T-shirts to accurately associate with its place of origin. Despite purposefully introducing potentially confounding variables, bacterial profiling-based soil identification from clothing maintains its viability for forensic analysis.

Reference(s):

1. Shokralla, S., Spall J.L., Gibson, J.F., and Hajbabeali, M. Next-Generation Sequencing Technologies for Environmental DNA Research. *Molecular Ecology.* 21 (2012): 1794–1805, accessed July 29, 2017. doi:10.1111/j.1365-294X.2012.05538.

2. Alyssa Badgley. Influences of Time, Temperature, and Quantity on Next-Generation 16s Bacterial DNA Profiles for Forensic Soil Evidence Analysis. (Master's thesis, Michigan State University, 2016).

Soil Identification, Bacterial Profiling, Next Generation Sequencing

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