Microbial Metagenomic Profiling Using Multi-taxa DNA Profiling and Bioinformatics to Discriminate Soil Specimens

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ABSTRACT

The analysis of soil can be an invaluable implementation for the forensic community due to it ubiquity and the vast array of information that can be obtained from both its biotic and abiotic content. Molecular techniques used to assay the biotic portion of soil have proven useful for soil characterization. Microbial metagenome profiling produces a unique soil biotic pattern, one that can be used to conceivably establish an evidentiary relationship between evidence soil and a crime scene. This research aims to expand on previously published data by applying microbial community profiling and bioinformatic tools to classify soil from the six different soil types, across the Miami-Dade County and establish a searchable soil DNA profiling database. For the purpose of this poster the soil samples were examined using multiplexed LH-PCR with only fungal and bacterial DNA markers however five taxa will be examined in total. The methods developed by this research will provide an efficient soil analysis tool and the database can be used to identify the geographic origin of an unknown soil sample based on its microbial DNA profile.

INTRODUCTION

Microbial metagenome profiling can produce a unique soil biotic profile or "DNA fingerprint" which can be used to differentiate that soil sample from all others. A previous study [1] from our lab was able to determine unique differences between soil types based on the biotic content even better than elemental analysis of the same soils. This research will determine whether soils from diverse geographical locations display distinct, reproducible DNA metagenomic profiles. The research will build on the previous publication by expanding the DNA analyzed to include five taxa, not only bacterial DNA but also fungal, nematode, archaeal and plant. Since soil type structures its intrinsic microbial community, soil samples will be collected from each of the six soil types (classified by USDA soil survey (http://websoilsurvey.nrcs.usda.gov/)) in Miami-Dade county, type 1:Urban Land-Udorthents, type 2: Lauderhill Dania-Pahokee, type 3: Rock Outcrop-Biscayne-Chekika, type 4: Perrine-Biscayne-Pennsuco, type 5: Krome and type 6: Perrine-Terra Ceia-Pennsuco [Figure 1]. The bioinformatic approach [2] will allow for the creation of a soil biotic profile database that can be queried when unknown samples are assayed. This research will increase the information content obtained and create a reproducible, and identifiable biotic DNA profile that will increase the power of discrimination and better distinguish soils from diverse locations, thereby increasing the resolution of a 'match'.

METHODS

Soil samples were collected from each of the six soil types [Figure 1] present in the Miami-Dade county, Florida in both the dry (January) and wet (July) season. The soil collection scheme is shown in figure 2; 100m transects of six subplots (1.5 m^2) and six soil samples from each subplot and only collecting the top 5 cm in a 2 cm diameter. DNA was extracted, quantitated and LH-PCR was performed with universal bacterial (27 forward labeled with NED™ and 355 reverse) and fungal (ITS5 forward labeled with 6-FAM™ and ITS2 reverse) primers. The PCR reagents and final concentrations were as follows; 1X reaction buffer, 2.5mM $MgCl_2$, 250 µm dNTPs, 0.1% BSA, 0.6µm primers, 1ng DNA, and 0.5 U DNA polymerase. A 9700™ thermocycler was used with the following parameters; an initial 11 minute denaturing step at 95°C, 25 cycles of denaturation at 95°C annealing at 52°C and extension at 72°C each for 30 seconds with a final extension at 72°C for 10 minutes. The PCR products were separated on a genetic analyzer and the data generated was imported into GeneMapper™ ID v4.0 and PrimerE *V6* software for analysis.

SAMPLING

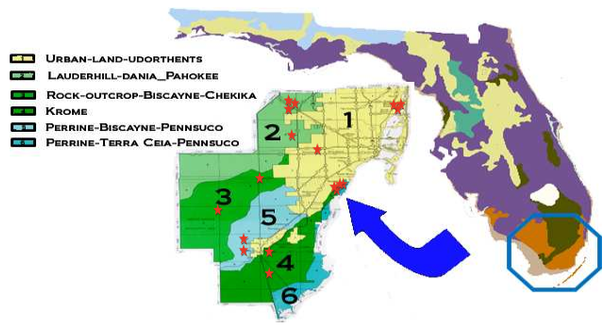

FIGURE 1: The six soil types of Miami-Dade County, Florida, the stars represent the 18 collection sites.

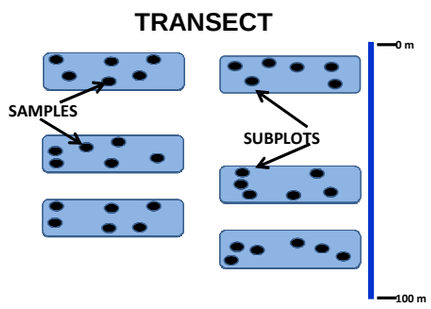

FIGURE 2: Soil collection transect (100m) showing the six subplots and the six samples per subplot used for each transect.

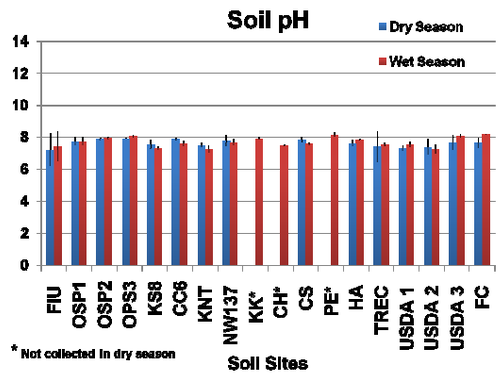

FIGURE 3: Soil pH in the wet and dry season collections.

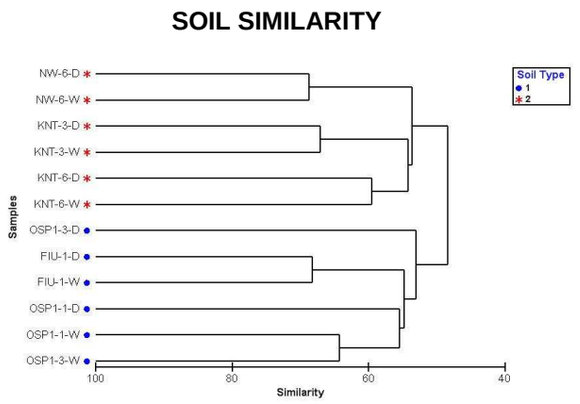

FIGURE 4: Dendrogram of microbial and fungal community data based on Bray–Curtis similarity coefficient for soil type one (blue circle) and two (red star) comparison.

FIGURE 5: The bacterial (341, 343, & 358) and fungal (219, 224, 315, & 317) amplicons which attribute to the dissimilarities between the soil type one and soil type two samples.

RESULTS AND CONCLUSIONS

A total of 18 soil transects have been collected encompassing all of the six soil types [Figures 1 & 2]. The changes in pH between the wet and dry season for each site [Figure 3] have been recorded and compared. The largest variation in pH within a soil type for the dry season had a standard deviation of 1.02 and a range of average pH among all sites of 7.2 to 7.90. For the wet season the average pH for most sites was greater than the dry season, with a standard deviation within a site of .92 and a range of average pH among all sites of 7.24 to 8.16. The pH has not been correlated to the soil DNA profiles. The pH did not change significantly between the seasons and remained mostly neutral which is expected due to the soil bedrock of limestone present in south Florida soils. For this poster 96 soil samples were analyzed with multiplexed LH-PCR using bacterial and fungal markers. 48 of the samples were from soil type one and 48 were from soil type two, encompassing both the dry (January) and wet (July) seasons [Figure 4]. The data obtained showed that the six samples from each subplot group together and seasonality did not affect the associations except for transect OSP1. The most obvious distinction from the preliminary data is that the two different soil types (one and two) can be differentiated based on their soil DNA profiles using only bacterial and fungal markers [Figure 4 & 5]. The distinctions can be attributed to the presence and absence of certain amplicons [Figure 5] and their abundance in each sample. The preliminary results are encouraging because with only two taxa (bacterial and fungal) we were able to distinguish soil samples by subplot, season, and soil type.

REFERENCES

[1] Moreno, L.I., D.K. Mills, J. Entry, R. T. Sautter, K. Mathee, 2006. Microbial metagenome profiling using amplicon length heterogeneity-polymerase chain reaction proves more effective than elemental analysis in discriminating soil specimens, J Forensic Sci 51:1-8.

[2] Yang C, Mills D, Mathee K, Wang Y, Jayachandran K, Sikaroodi M, et al. An eco-informatics tool for microbial community studies: supervised classification of Amplicon Length Heterogeneity (ALH) profiles of 16SrRNA. J Microbiol Methods 2006;65:49–62.

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