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

Maria Angelica Mendoza, PhD.1 DeEtta K. Mills, PhD.1
1. Department of Biological Sciences, Florida International University
Miami, FL 33199

ABSTRACT

The analysis of soil can be an invaluable implementation for the identification of microbial communities due to its complexity 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 biologist pattern, one that can be used to concurrently establish an explanatory relationship between evidence soil and crime scenes. 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 workable soil DNA profiling database. For the purpose of this poster, the soil samples were examined using multiplexed L1H-PCR with only fungal and bacterial DNA markers for the six wells and were examined in triplicate. 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 microbial DNA profile.

INTRODUCTION

Microbial metagenome profiling can produce a unique soil biota 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 loci, not only bacterial DNA but also fungal, nematode, archaean 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-Usdarm, type 2: Lafiulah-Dani-Pahokee, type 3: Rock: Outcrop-Blange-Quanah, type 4: Perrine-Brazaine-Pennasco, type 5: Krome and type 6: Perrine-Terra-Ciada-Pennasco [Figure 1]. The bioinformatic approach [2] will allow the creation of a soil profile database that can be queried when unknown samples are assessed. 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 this method.

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 m²) and six soil samples from each subplot and only collecting the top 5 cm in a 2 cm diameter. DNA was extracted, quantitated and L1H-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 MgCl₂, 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 15 minutes. The PCR products were separated on a genetic analyzer and the data generated was imported into GeneMapper™ v4.0 and PrimerE V6 software for analysis.

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 soil type has 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 0.82 and a range of average pH among all sites of 7.24 to 7.16. 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 L1H-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 soil 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


ACKNOWLEDGMENTS

We would like to thank the Central Intelligence Agency (CIA) (HM1582-09-0011) for funding this project as well as all the locations and personnel who have allowed us to collect soil for our research.

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.