Determination of host-associated bacterial communities in the

rhizospheres of maize, acorn squash, and pinto beans

The rhizosphere of a plant is the soil surrounding the plants roots and is a major component of the host plant’s host-associated microbial population [1]. The relationship between the host and much of this microbial community is symbiotic to varying degrees [2]; the health of the host is may depend on the utilization of the nutrient processing or infection resistance capabilities of the microbes, and the microbes may depend upon the environment provided by the host for reproduction. One of the most studied symbioses, for instance, is that of the relationship between nitrogen fixing bacteria such as Rhizobia and host legumes [3]. Several studies have attempted to map host-associated bacterial populations with the health of the host plant as it relates to a particular infection in order to glean which bacteria are able to mitigate the infection [4,5]. An understanding of that sort of relationship could potentially lead to methods for disease control based on the introduction of helpful bacteria rather than the use of chemicals that can cause harmful collateral environmental damage.

16S Ribosomal RNA has long been used for bacterial speciation. This region is highly conserved within a species and even among species of the same genus. Due to this low level of variability a single set of restriction enzymes can be used to generate probes for PCR sequencing that will work across a broad range of bacteria. Additionally, the high concentration of ribosomes present in cells provides a large amount of material to work with. This method has been used for more than a decade and there is already a large library of 16S sequences documented for various organisms [6].

Classic approaches to PCR involve amplification of the genetic material of a sample in a culture grown in a laboratory. However, it has long been known that the vast majority of bacteria do not grow under known laboratory conditions. The reasons for this are mainly speculated to be either that certain stress conditions (such as in an unfavorable environment) repress certain cell’s tendency to replicate, or that the bacteria are host-associated and require the symbiosis for replication [6]. The result is that culture-based experiments for microbial speciation miss the vast majority of the species involved. Therefore, in order to identify the bacterial communities of host-associated genomes which are, almost by definition “unculturable”, 16S identification is best performed in situ – that is, in something very close to a natural environment. PCR methods are used to amplify probes for the 16S region using a set of restriction enzymes common for that rRNA region in bacteria. The probes are then hybridized with the sample in situ [4,6]. There have already been several studies that have used in situ PCR based methods such as 16S rRNA clone library analysis for rhizosphere bacterial analysis which have contributed to the knowledge base, but this still comprises only a tiny fraction of the rhizospheres which may be of interest.

Experiment:

The purpose of this experiment is to examine the differences in bacterial populations between various related and unrelated rhizospheres, as well as compare those to a biome lacking in any of the host plants to find the bacterial “background noise”. A garden bed will be prepared beforehand and an initial, host-free analysis will be performed to acquire base bacterial soil content. Environmental variables such as nutrient levels, moisture content, pH, salinity, and temperature will be taken and recorded as well. Although these factors are not considered within the scope of this experiment, the data may be useful for reference when comparing this study to other, similar ones.

Seven individual beds will be planted using maize, acorn squash, and pinto beans – a standard “three sisters” mezzo American agricultural technique. Three beds will be planted with only one crop. In between each of those beds will be a bed that has the two neighboring crops planted together. In the center will be the bed with all three. 16S analysis will be performed individually on large samples from each bed gathered at harvesting time.

In each step, an initial soil sample will be taken and used as a template for PCR. A primer set which should allow for the generation of probes that will work for a large portion of the bacterial population – possibly 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R (5’-TACGGTTACCTTGTTACGACTT-3’) - will be used. Amplification will be performed by M13 insertion and growth in e. coli in a medium that will tag the probe, such as 32P [4]. The probes will then be hybridized on large soil samples to construct the eight 16S rRNA libraries.

The resulting libraries will be compared against national databases first. Then the relative phylogenic content of each will be compared to see how the presence of multiple rhizospheres affects the bacterial profile. This bacterial population information and the development of the methodology for obtaining that information are the goals of the experiment; applicability of that information is for later studies.

Discussion

This experiment could potentially lay the groundwork for larger projects. In order to get useful host-associated information, an experiment such as this would need to be conducted with a variety of different environmental variables; the same data could be gathered for the same plant rhizospheres, but using soil with different composition and base bacterial content and in different climates. A single bed could be used for several seasons to observe changes in microbial content. Bacteria could be introduced to the environment to find out how it incorporates. Other elements, such as charcoal, might be added to soil to contribute to the efforts in reverse engineering terra preta. Observations of plant health and crop yield of the plants could be conducted to determine optimal growing environments and disease resistance potential.

 16S PCR analysis is time consuming and complicated. With the advent of high-throughput sequencing, it is possible to acquire the entire metagenomic content of a sample (given a large enough sample). It may be possibly to pick out identifiers in the 16S region to create the libraries without primer amplification and hybridization. There have been several attempts to accomplish this using methods such as GAST and naïve Bayesian classifiers. Unfortunately, high-throughput sequencing results in small fragments – generally less than 100 bases – so the methods are not yet reliable [8]. It may be possible to use this experimental environment to gauge the effectiveness of various analysis techniques by sequencing samples from the same bed as that used for PCR analysis and finding how close to the PCR results the methods come.

References

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