Determination of host-associated bacterial communities in the

rhizospheres of maize, acorn squash, and pinto beans

Eukaryotes are host to enormous microbial communities. About 90% of some animal’s cells are actually single celled organisms living in symbiosis with the host rather than their own eukaryotic cells. With plants, much of the host-associated microbial community actually inhabits the soil about the roots. This area is called the rhizosphere [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.

The 16S locus of ribosomal RNA has long been used for purposes of identifying organisms. 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 taxa. 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]. With the proper choice of primers, it is possible to identify a large swath of bacteria or pick out a narrower range with more specific primers. Gregoris at al performed a study that gauged numerous taxa-specific 16S bacterial primers by specificity and universality [7] that could be used to choose appropriate primers for bacterial tagging.

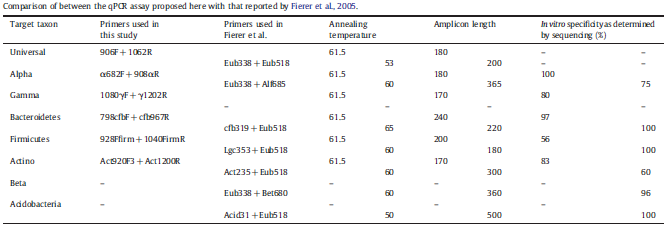
Classic approaches to PCR involve amplification and hybridization in cultures grown in a laboratory. However, it has long been known that the vast majority of bacteria do not grow under known laboratory conditions. The laboratory conditions evidently do not provide the environment needed for the bacteria to propagate [6]. The result is that culture-based experiments for microbial speciation miss the majority of the species involved. Therefore, in order to identify the bacterial communities of host-associated genomes which are, almost by definition “unculturable”, the hybridization phase of 16S PCR analysis 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 tagged, often by fluorescence, 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 samples will be taken and used as a templates for PCR. Primers will be selected based upon the work of Gregoris[7] and the earlier work of Fierer[8] referenced in that article. The “universal” primer set will be used to estimate total bacterial populations in order to normalize a relative quantitative PCR analysis. Amplification of the probes will be performed by M13 insertion and growth in e. coli in a medium that fluorescently label the probes. The probes will then be hybridized in situ on large soil samples. Relative quantities of bacterial taxa can then be measured by fluorescent microscopy and/or flow cytometry to construct the eight 16S rRNA libraries. This process is well established and known as FISH (Fluorescent In SituHybridization).



The relative phylogenic content of each will be compared to see how the presence of 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 [10]. 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

[1] A.D. Kent, E.W. Triplett, Microbial Communities and their Interactions in Soil and Rhizosphere Ecosystems, Annu. Rev. Microbiol. 56 (2002) 211-236

[2] T. Bisseling, J.L. Dangl, Next-generation Communication, Science 324 (2009) 691

[3] N.J. Brewen, M.J Ambrose, Root Nodules, Rhizobium and Nitrogen Fixation, Biotechnology in Agriculture Series; Peas: Genetics, molecular biology and biotechnology  Book Series: Biotechnology in Agriculture Series    Volume: 10      Pages: 237-290    Published: 1993

[4] Y.B. Zhu, J.Q Tian, Rhizosphere bacterial communities associated with healthy and Heterodera glycines-infected soybean roots, EUROPEAN JOURNAL OF SOIL BIOLOGY 58 (2013) 32-37

[5] R. Mendez, M. Krujit, Deciphering the rhizosphere microbiome for diseasesupressive bacteria, Science 332 (2011) 1097-1100

[6] W. Liesack, E. Stackebrandt, Unculturable Microbes Detected by Molecular Sequences and Probes, BIODIVERSITY AND CONSERVATION 1:4 (1992) 250-262

[7] T.B. Gregoris, N. Aldred, Improvement of phylum- and class-specific primers for real-time PCR quantification of bacterial taxa, Journal of Microbial Methods 86:3 (2011) 351-356

[8] N. Ferrier, J.A. Jackson, Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays, Appl. Environ. Microbial. 71 (2005) 4117-4120

[9] H.J. Miller, G. Henken, Variation and composition of bacterial populations in rhizospheres of maize, wheat, and grass cultivars, Can. J. Microbiol. 35 (1989) 656-660

[10] O. Mizrahi-Man, E.R. Davenport, Taxonomic Classification of Bacterial 16S rRNA Genes Using Short Sequencing Reads: Evaluation of Effective Study Designs, PLOS ONE 8:1 (2013)