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 primers can select and amplify the 16S region across a broad range of taxa. 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]. There are numerous ways of identifying particular 16S sequences, one of which is quantitative PCR.

One qPCR method –TaqMan - uses fluorescent probes that select for particular sequences within the region being amplified using the primers. The probes do not fluoresce when intact due to energy transference between the reporter and quencher ends. During amplification, however, any probes that have hybridized to a matching strand release the reporter which can then be detected. The volume of the fluorescence detected produces an asymptotic curve which can be used to deduce the relative amount of the organism detected for.

This experiment will use TaqMan qPCR to measure relative bacterial content of soil samples taken from rhizospheres and compare them with one another and against samples taken without any of the targeted hosts in order to determine which bacteria are associated with the hosts.

Experiment:

Samples will be taken and analyzed before any target crops are planted in order to find relative bacteria present without any of the host-association sought. 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]. These are fairly generic primers that will only provide general taxa, but each can be labeled with a different fluorescent marker to allow them to all be run in a single sample. More specific primers can be selected for more granular identification within each of the general taxa.



The relative phylogenic content of each will be compared to see how the presence of rhizospheres affects the bacterial profile. It is of interest not only how a single crop’s rhizosphere may affect the bacterial profile, but how the bacterial content changes when rhizospheres of plants known to complement one another’s health are combined. 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. Observations of plant health and crop yield of the plants could be conducted to determine optimal growing environments and disease resistance potential. 16S qPCR 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). The total metagenome of the samples would provide much more granular and useful data.

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)