Molecular Analysis of Turfgrass Rhizosphere Bacterial Communities

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Objectives:

1. To determine the bacterial taxa associated with turfgrass soils in an attempt to correlate soil quality parameters with particular microbial taxa.

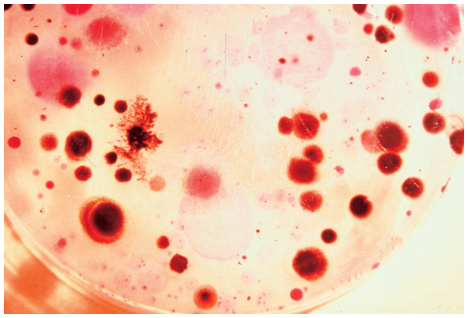
Start Date: 2001
Project Duration: three years
Total Funding: $90,000

Microbial characteristics of soils and plant rhizospheres are being viewed increasingly as sensitive indicators of soil health since there are clear beneficial relationships between microbial diversity, soil and plant quality, and ecosystem sustainability. In turfgrass management, soil health issues are becoming more important as golf course superintendents are forced to manage turfgrasses under less-than-ideal agronomic practices.

Research at Cornell University is combining traditional culturing methods with new molecular methods for characterizing microbial communities. An advantage of the combined approach is that many non-culturable microorganisms known to exist in all soils can now be studied. Some of these non-culturable microorganisms may have major impacts on soil quality and impacts on turfgrass quality.

The goal of this research is to understand which microorganisms inhabit turfgrass soils so that we can correlate soil quality parameters with specific microbial taxa. Such a molecular approach for studying microbial communities is being used widely in microbial ecology and revealing novel microorganisms and microbial associations with plants. This work involves the extraction of microbial DNA from microbial cells in soil, the amplification of specific DNA sequences from this DNA that serve as signatures for particular microorganisms, and matching the signatures with known sequences that have been studied in the past.

Our work is focusing on microbial communities inhabiting soil-based and sand-based rootzone profiles under L-93 creeping bentgrass. Work in 2002 continued to refine methods development and efficient DNA extraction protocols were developed. Every sample requires specific methods development since many factors influence the ability to extract DNA from various soils as well as the ability to manipulate the DNA subsequent to soil extraction.



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From the DNA samples extracted from the sand rootzone and the soil rootzone, we amplified 16S rRNA genes that serve as useful signatures for microbial species. These amplified genes were cloned into *E. coli* and individual clones picked from plates and stored. Each clone theoretically represents a unique 16S rDNA sequence. Over 350 clones from each of the soil samples were collected.

To verify the uniqueness of the individual clones, the inserts were subjected to restriction analyses in which the inserts were excised from the *E. coli* plasmid and digested with restriction enzymes. Those inserts with a unique banding pattern represent unique 16S rDNA sequences. A greater diversity of sequence types were found in the sand rootzones than in the soil rootzones. However, the diversity of clones will be verified after sequence analysis.

Unique clones were submitted to the Cornell Bioresource Center for sequence analysis. The sequence analysis provided information on the specific nucleotide sequence of the 16S rRNA gene. These sequences were then subjected to BLAST searches to identify the bacterial species to which the sequence belongs.

Not surprisingly, after sequencing over 40 clones, nearly all so far match other bacterial sequences that have been previously described, but to which no species designation has yet been ascribed. This is common with many soil microbial analyses where the vast majority of microorganisms that can be detected have not previously been studied in culture. As a result, their species affiliations can only be inferred from a phylogenetic analysis, which we will be conducting in 2003

In 2003 we hope to complete the sequencing parts of the project and we will generate phylogenetic trees of the 16S rDNA clones from each of the two rootzones. We also have begun to develop a collection of culturable bacteria from each of the two rootzones. We will be generating 16S rDNA sequences for these strains.

Summary Points

- We have successfully extracted bacterial DNA from turfgrass soils and amplified 16S rRNA genes from these DNA samples.
- Sequence analysis has revealed that the vast majority of bacterial species recovered thus far represent previously described but unculturable bacteria.
- In 2003, the sequencing parts of the project will be completed and phylogenetic trees of the 16S rDNA clones from each of the two rootzones will be generated and compared.
- A collection of culturable bacteria from each of the two rootzones is being developed and 16S rDNA sequences for these strains will also be generated.