Molecular Analysis of Turfgrass Rhizosphere Bacterial Communities

Eric B. Nelson
Cornell University

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: two years
Total Funding: $60,000

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 communities, 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 conditions.

Research at Cornell University is taking advantage of new molecular methods for characterizing microbial taxa potentially involved in the maintenance of healthy soils. An advantage of such a molecular approach over the traditional culturing approach is that microbes such as anaerobes, autotrophs, heterotrophic oligotrophs, and chemolithotrophs that have not been cultured previously can be investigated.

Some of these non-culturable microorganisms may have major impacts on soil quality and impacts on turfgrass quality. By combining this molecular approach with advanced fluorescence-microscopy techniques, both qualitative and quantitative ecology of non-culturable microorganisms in turfgrass soils can be studied.

The goal of this research is to determine the bacterial taxa associated with turfgrass soils in an attempt to correlate soil quality parameters with particular microbial taxa. We are following an experimental approach that combines traditional culture-dependent methods with new and developing molecular methods for characterizing microbial taxa potentially involved in the maintenance of healthy soils.

The need for the combined approach is based on the observations that less than one percent of the microorganisms observed in soils through direct microscopic examination are culturable using conventional laboratory cultivation techniques.

An advantage of the molecular approach we have chosen over the traditional culturing approach is that we will be able to investigate microbes such as anaerobes, autotrophs, heterotrophic oligotrophs, and chemo-lithotrophs that have not been cultured previously.

Our work in 2001 demonstrated the feasibility of molecular methods of characterizing microbial communities in turfgrass soils. We have worked out a number of the technical obstacles to this work, but feel there are still a number of stumbling blocks regarding the interpretation of the sequence information.

In 2001, our work centered on several different areas: 1) improving extraction of microbial DNA from turfgrass soils, 2) developing the PCR and cloning aspects of the work, and 3) initial attempts at characterizing 16S rDNA clones. For our initial work, samples were taken from a soil-based putting green at the Cornell Turfgrass Field Research Facility.

Additional samples were also taken from perennial ryegrass/tall fescue plots also at the Turf Field Facility. Subsequent work will be done largely on microcosms using soil collected from two widely divergent rootzone profiles where we will conduct a more detailed analysis and verify the utility of our approach.

Extraction of DNA from soil habitats represents special challenges due to the nature of the soil matrix, the nature of the organisms residing in soils, and the procedures necessary for efficient DNA extraction.

In 2002, we plan to further develop this methodology, working primarily with soil microcosms, examining two typical rootzone profiles (a USGA-based profile and a soil-based profile). These will be collected from an area golf course and brought back to the laboratory for analysis. We plan a full-blown analysis of these soils using the cloning procedures we have already developed.

We further plan to take advantage of new genomics technologies for characterizing the collective genomes of soil microorganisms. This will involve the creation of bacterial artificial chromosome (BAC) libraries of soil DNA. These libraries can be screened for 16S rRNA sequences and then the entire clone sequenced, providing a phylogenetic anchor from which flanking functional and regulatory genes might be identified, thus linking species with a particular function.

We fully anticipate that by the end of 2002, we will have a complete phylogenetic tree of microorganisms in the two rootzone profiles, and we will have developed our BAC technology where we can begin a similar analysis in 2003.

Summary Points

- Researchers demonstrated he feasibility of molecular methods of characterizing microbial communities in turfgrass soils.
- In 2002 , they plan to further develop this methodology, working primarily with soil microcosms, examining two typical rootzone profiles (a USGA-based profile and a soil-based profile).
- Researchers are planning to created bacterial artificial chromosome libraries of soil DNA which may lead to the creation of a complete phylogenetic tree of microorganisms in the two rootzone profiles.