Promotion of Turf Health through Early Pathogen Detection: Development of a Turf PathoCHIP

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Objectives:

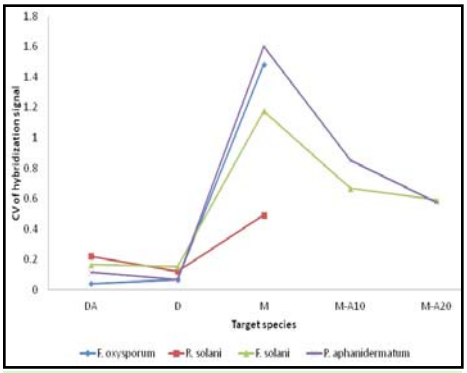
To develop and implement a highly sensitive DNA macroarray system,"Turf PathoCHIP", for rapid detection of known and emerging turfgrass pathogens based on the internal transcribed spacer sequences of the rRNA genes that are used for DNA barcoding of fungi.

Start Date: 2011
Project Duration: 3 years
Total Funding: $60,000

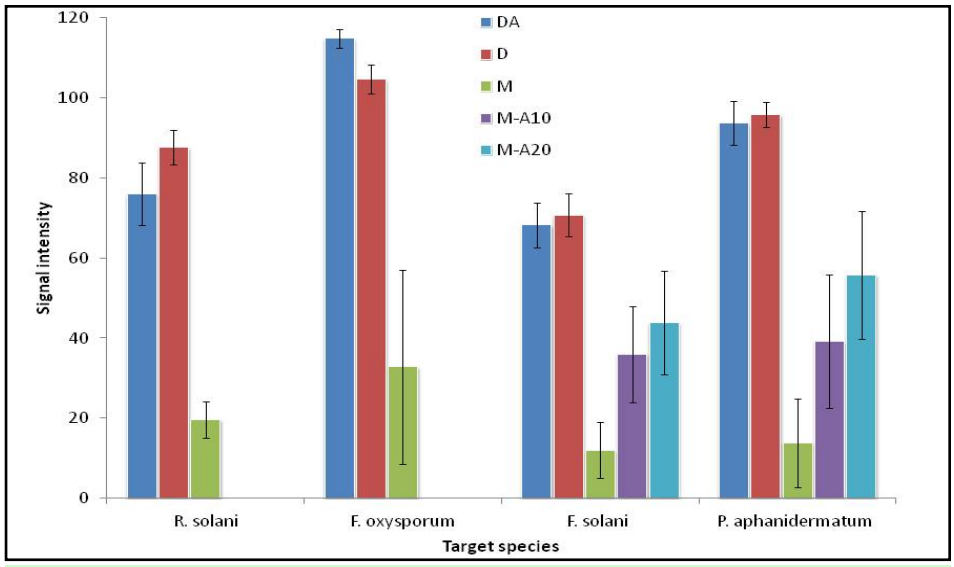
Early detection and rapid pathogen identification is essential for turf disease management. Fungi constitute the majority of pathogens that infect and damage turfgrasses. Over two hundreds fungal and fungus-like species have been recognized as turf pathogens, many of which are understudied. Identification of turfgrass pathogens poses a challenge because different pathogens may infect the same host concurrently and may produce similar symptoms. Traditionally, turfgrass diagnosticians use direct observations or culturing of pathogens from diseased plant samples to make a diagnosis.

DNA PathoCHIP is molecular technique that offers a fast, culture-independent alternative for the diagnosis of turf pathogens from field samples. The advantage of the technique is its remarkably high throughput compared to other detection methods. Hundreds of different pathogens can be simultaneously detected with one array in one reaction within a few hours.

We initiated a study in 2009 to optimize the technique for use in detection of turfgrass pathogens. The goal was to develop a novel technical approach that could increase the sensitivity of a PathoCHIP to enhance its early pathogen detection power, while maintaining the detection specificity to ensure accurate pathogen identification. Probes tested in a pilot study were based on four important pathogens of turfgrass and other plants: *Rhizoctonia solani* (basidiomycete), *Pythium aphanidermatum* (oomycete), *Fusarium solani* (ascomycete) and *F. oxysporum* (ascomycete) that cause brown patch, Pythium blight, root and vascular diseases, respectively.



Coefficient of variation of hybridization signal intensity among different probe types for the four target species.



Signal intensity comparison of different oligonucleotide probe types for the four species tested. The data are means ±SD (n =12) except F. oxysporum *where n=6.*

The dimeric oligonucleotide probes provided a low measurement variation and superior signal intensity. The new technique was remarkable in detecting low quantities of pathogen, which was a thousand times more sensitive than the PCR detection technique. The method was also successfully validated with target species infected turfgrass or soil materials vis-à-vis disease free materials.

We also embarked on a sample collection exercise for important diseases of turfgrass and microbes co-inhabiting in turfgrass. Great progress has been made in this area. We have collected over 200 pathogen and pathogen strains, sequenced the ITS region of the rRNA gene (for probe design) and fully identified the pathogen to species and subspecies level. The purpose of this exercise is to use the ITS sequence to design sequence specific probes for all important pathogens infecting turfgrass.

Starting 2011, different methods (bioinformatics software based and direct visualization) are being devised on how to detect the signature probes. This process requires all known related species sequence to be aligned and compared with sequences of target pathogen.

Pathogen specific probes will be designed and printed into an array (PathoCHIP) and thereafter validated for use by means of non-target pathogen, symptomatic and symptom-free target plants. The process will also be validated with microscopy, culturing, and real-time PCR.

Summary Points

- A database of more than 200 turfgrass pathogenic fungal strains have been built. All pathogens have ITS region sequence and have been preserved in various forms.
- Design of probes specific to target pathogens of turfgrass is underway.
- The PathoCHIP (array) system has been optimized for sensitivity.