Accurate Identification and Gene Expression in Relation to Virulence of Rhizoctonia *Isolates Infecting Turfgrasses*

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Objectives:

1. Molecular identification of *Rhizoctonia solani* isolates pathogenic to turfgrasses using Universally Primed-Polymerase Chain Reaction (UP-PCR) and nucleic acid hybridization analysis.
2. Expressed Sequence Tag (EST) analysis for surveying genes and creating a gene database for *R. solani* with emphasis on genes affecting virulence and pathogenicity.

Start Date: 2007
Project Duration: three years
Total Funding: $90,000

Rhizoctonia blight (brown patch), caused by *R. solani* is a disease of cool-season grasses, including bentgrasses, fescues, and ryegrasses. Anastomosis groups (AG) 1, 2, 4, and 5 have been previously isolated from blighted grasses. *Rhizoctonia* leaf and sheath spot of both warm- and cool-season grasses are caused by both *R. zeae* and *R. oryzae*. *Rhizoctonia* species and AGs are reported to differ in sensitivity to common fungicides. There are non-pathogenic binucleate *Rhizoctonia* isolates, some of which are antagonists of the pathogenic isolates of turfgrass and have been used in the biocontrol of the pathogen.

The prevalence and severity of *Rhizoctonia* diseases on turfgrasses depends, among other factors, on infection by a particular species and AG of *Rhizoctonia*. Thus, minimization of chemical use as well as consistent and reliable management of *Rhizoctonia* diseases with genetic and biological methods will largely depend on identification of *Rhizoctonia* isolates to species and subspecies level and knowledge of its virulence-regulating genes.

Identification of *R. solani* to anastomosis group (AG) level is commonly based on the ability of an isolate to anastomose with tester strains. Some isolates fail to anastomose because of genetic instability or environmental or nutritional conditions, and this testing is relatively time-consuming and often confusing. Anastomosis grouping may not be the best indicator of genetic relatedness. We plan to create genome fingerprint of various *Rhizoctonia* isolates using UP-PCR (Universally Primed-Polymerase Chain Reaction) and AFLP (Amplified Fragment Length Polymorphism) and correlate with type isolates at the genus and anastomosis group levels.

Dr. Dilip Lakshman and his colleagues are using molecular techniques to create genome fingerprints of various Rhizoctonia *isolates.*

Molecular markers will be developed to detect and distinguish isolates of different AGs and to differentiate pathogenic from non-pathogenic isolates. Multilocus genome sequencing with ribosomal DNA, beta-tubulin, and other genes will also be compared for phylogenetic purposes. The advantages of genome fingerprinting over rDNA sequencing is that SCAR (Sequence Characterized Amplified Regions) markers are most likely identifiable with the former approach.

In a collaborative study involving Dr. Brandon Horvath, we initiated a survey of *Rhizoctonia* diseases of turfgrasses in the state of Virginia for molecular characterization. A VT graduate student, Mr. Sajeewa Amaradasa, collected diseased samples from creeping bentgrass and annual bluegrass golf course greens, and from tall fescue on a home lawn, a road-side area, and a sod farm.

After microscope confirmation that each isolate was *Rhizoctonia*-like, the colony morphology was examined to determine species. Suspected *R. zeae* were confirmed by lactophenol test. There were a few isolates collected from tall fescue having morphological features similar to *R. cerealis* which will be confirmed by nuclear staining. We collected 93 isolates of *R. solani* and 10 isolates of *R. zeae* from Blacksburg and Richmond in fall 2007. In addition, Dr. Horvath has in his collection about 115 *R. solani* isolates and 13 *R. zeae* isolates from various locations of Virginia (Blacksburg and Richmond), Georgia (Griffin), South Carolina (Pee Dee ARES), and Wisconsin (Blackwolf Run & Whistling straits).

An Expressed Sequence Tag (EST) analysis of *R. solani* (AG-4) isolate Rs23 infecting ornamentals was initiated to compare and identify pathogenically associated *Rhizoctonia* genes. AG-4 isolates cause diseases in a broad range of hosts, including ornamentals and turfgrasses. Two normalized EST libraries specific to a virulent isolate and a 3-O-methyl glucose-induced virulence-repressed isolate of *R. solani* have been constructed. About 1,000 EST clones from each library (total 2,000 clones) have been sequenced so far, and an overall analysis of the cDNA sequences will be soon carried out.

Summary Points

- *Rhizoctonia* isolates have been collected from creeping bentgrass and annual bluegrass golf course greens and from tall fescue on a home lawn, a road-side area, and a sod farm from several states. Initial morphological and microscopic characterization is in progress.
- Two EST libraries from a virulent *R. solani* (AG-4) isolate Rs23 and its chemically induced virulence-suppressed form have been constructed. About 1,000 EST clones each from the virulent isolate and 3-O-methyl glucose-induced virulence-repressed isolates of *R. solani* (AG-4) have been sequenced. Analysis and initial characterization of expressed genes are in progress.