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

Dilip K. Lakshman
USDA-ARS

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.

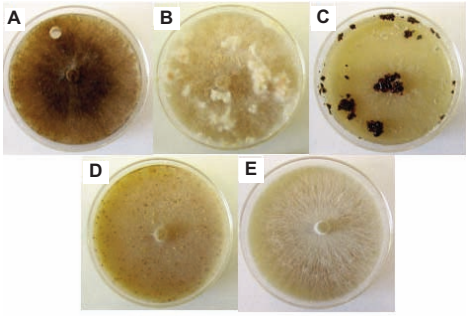
Start Date: 2007
Project Duration: three years
Total Funding: $90,000

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. Also, 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.

During the summer of 2008, we collected more *Rhizoctonia* samples from Northern Virginia and Beltsville area of Maryland. We were able to collect 136 isolates from Leesburg and Woodbridge of VA, and 83 isolates from Beltsville, MD. Dr. Brandon Horvath and his research team also helped in collecting additional 22 samples from Richmond and Virginia Beach. The total *Rhizoctonia* isolates we have at Beltsville USDA-ARS, so far, amounts to 448. Out of that, around 7% of isolates belong to either *R. zeae* or *R. oryzae*. The nuclear staining of suspected *R. cerealis* isolates from Blacksburg (collected in 2007) proved to be *R. solani* since all of them were multi-nucleate.

The molecular identification of *Rhizoctonia* isolates will be carried out by employing AFLP, UP-PCR, and sequencing of rDNA-ITS regions. Sequencing ITS regions will be utilized to group sample isolates together with tester strains in order to come up with their phylogenetic relationships. The banding patterns of UP-PCR and AFLP will be used to construct phenograms of the tested isolates. Similar to the analysis of ITS regions, the bands generated will be analyzed with the results of tester trains to find differences at molecular level.



*Differences in colony morphology of a few isolates after 10 days incubation on PDA. Isolates A (*R. solani *AG2-1), B (*R. oryzea*), C (*R. solani *AG1-1A), D (*R. zeae*) and E (*R. solani *AG4) were collected either from Richmond, Beltsville, or Leesburg area.*

Out of the total isolate collection, we selected a random sample of approximately 10% encompassing different geographic locations. The colony morphology of these isolates was recorded after 10 days incubation on PDA. The color of mycelial mat and color and size of sclerotia of the isolates differed according to their species and anastomosis group (AG). The average hyphal diameter of all the isolates were within 5-11µm which agrees with the previous published data for *R. solani*, *R. zeae,* and *R. oryzae*.

Presently, we are extracting DNA from the selected isolates for carrying out molecular detection techniques. Simultaneously, we are planning to perform hyphal fusion experiments with tester strains on water agar plates in order to determine AGs of the isolates. The research work carried out previously has recorded the AGs of 1, 2, 3, 4, 5 and 6 from diseased turfgrasses. However AG1, AG2-2 and AG4 are the most common. The data generated from molecular techniques will be considered for accurate identification of brown patch causal agents into their species and intraspecific level.

We are also investigating the pathogenicity of *R. solani* through Expressed Sequenced Tag (EST) analyses. Approximately 1,000 clones each from two virulence-differentiated EST libraries have been sequenced and analyzed. The number of unigenes identified from virulent and avirulent libraries are 214 and 590, respectively. We are conducting a rudimentary analysis of genes under the two virulence differentiable conditions. However, as in any gene expression project, we need to sequence more ESTs to make a comprehensive bioinformatics analysis of the two EST libraries to identify up- or down-regulated genes related to virulence.

Summary Points

- We have collected a total of 448 *Rhizoctonia* isolates from golf courses and lawns of Virginia and Maryland. Out of them, around 7% of isolates belong to either *R. zeae* or *R. oryzae*. The colony morphology, color of mycelium, color and size of sclerotia of a random sample of 10% of those isolates have been characterized so far.
- We have conducted initial experiments with UP-PCR technique and amplification of ITS regions and selected the primers for future full-scale experiments.
- From the initial analysis of the two EST libraries thus far, about 214 and 590 unigenes have been identified from the virulent and avirulent EST libraries, respectively.