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

Dilip K. Lakshman
USDA-ARS

Objectives:

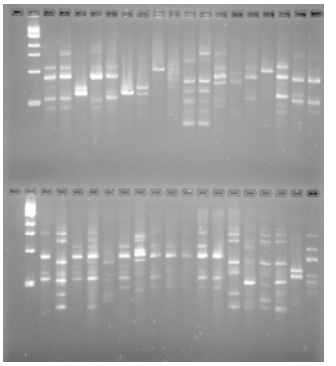
1. Molecular identification of *Rhizoctonia solani* isolates pathogenic to turfgrasses using Universally Primed-Polymerase Chain Reaction (UP-PCR) and nucleic acid hybridization analysis.
2. Expressed Sequence Tag (EST) analysis for surveying genes and creating a gene database for *R. solani* with emphasis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

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

Rhizoctonia blights, variously named as brown patch, large patch, yellow patch, leaf and sheath spots, and brown ring patch, are caused by *R. solani* sensu lato. Symptoms are mostly determined by the isolates of the pathogen, species of turfgrass, and climatic conditions. For example, brown patch is a disease of cool-season grasses in the summer, caused by multinucleate *R. solani* (Tel: *Thanatephorus cucumeris*) isolates. Anastomosis groups (AG) 1, 2, 3, 4, 5 and 6 have been isolated from blighted grasses. Leaf and sheath spots in both cool- and warm-season grasses are caused by multinucleate *R. oryzae* and *R. zeae* (Tel: *Waitea circinata* var *oryzae/zeae*) in the summer. Yellow Patch of both cool- and warm-season grasses are caused by binucleate *R. cerealis* (Tel: *Ceratobasidium cereale*) AG-D isolates. Recentlly a new disease, Brown ring patch, has been reported to be caused by *W. circinata* var *circinata*.

Rhizoctonia species and AGs are genetically heterogeneous groups and reported to differ in sensitivity to common fungicides and host susceptibility. The prevalence and severity of *Rhizoctonia* diseases on turfgrasses depend, among other factors, on infection by a particular species or AG (anastimosis group) of *R. solani*. 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 levels and knowledge of its virulence-regulating genes.

In a team effort with Dr. Brandon Horvath and Sajeewa Amaradasa, we collected a total of 448 *Rhizoctonia* samples from 5 locations in Northern Virginia and Maryland during the summer of 2008 and 2009. We selected a random sample of approximately 10% of the collected isolates for further studies. Isolates were grouped into species level using conventional approaches. AG group of each isolate was determined by anastomosis behavior with tester strains.



UP-PCR genome fingerprints of 38 isolates of Rhizoctonia *species. Genomic DNA of the isoaltes were amplified with primer L15/AS19 to obtain banding profiles.*

Genomic DNAs isolated from *Rhizoctonia* isolates are being analyzed employing molecular techniques like sequence analysis of the ribosomal DNA internal transcribed spacers (rDNA-ITS) regions and Universally Primed Polymerase Chain Reaction (UP-PCR) to group the *Rhizoctonia* isolates and compare them with conventional grouping methods. rDNA-ITS PCR products were sequenced and homology searched with rDNA-ITS sequences from the NCBI public database.

Analyzing rDNA-ITS sequence variabilities, we found that of the 54 isolates tested, 33 (61%) isolates belong to *R. solani*, 9 isolates (17%) belong to binucleate *Rhizoctonia*-like fungi, and 12 isolates (22%) belong to *R. zeae/W. cercinata* var *circinata*. Of the 33 *R. solani* isolates, 20, 12 and 1 belonged to AG2-2IIIB, AG 1-1B and AG-5, respectively.

UP-PCR products will be cross-hybridized to evaluate the possibility of developing a dot-spot hybridization detection method. Also, we will look for Sequence Characterized Amplified Region (SCAR) markers in the UP-PCR.

First time for any *R. solani* AG-4 isolate, we are investigating the expressed gene profiles through Expressed Sequenced Tag (EST) analyses of two virulence-differentiated and rDNA-normalized EST libraries (Objective 2). Approximately, 1,110 clones from each of the two libraries (total 2,220 cDNA clones) have been sequenced, generating 742 and 842 high quality EST sequences and 532 and 495 unigenes, respectively. Among the important genes identified from the two libraries are those involved in plant cell wall degradation, fungal cell wall protection, disease elicitation, avoidance of host and drug resistance, cellular metabolism, transport, division, and mating.

Summary Points

- We have collected a total of 448 *Rhizoctonia* isolates from golf courses and lawns of Virginia and Maryland. Analyzing the rDNA-ITS sequence variations of 54 randomly sampled isolates, we found that majority (i.e., 61%) of the isolates belong to *R. solani*, while 22% belong to *R. zeae/W. cercinata* var *circinata,* and 17% belong to *R. cerealis*/*Rhizoctonia*-like fungi. Of the identified *R. solani* isolates in this study, majority belong to AG2-2IIIB and AG 1-1B.
- Genome fingerprinting of *Rhizoctonia* isolates using UP-PCR and AFLP techniques are in progress.
- Analyzing the two virulence-differentiated EST libraries, we have identified 532 and 495 unigenes of *R. solani* AG-4. Among several identified genes are those with possible roles as elicitors of disease, in cell wall degradation, avoidance of host and drug resistance and mating.