A Biocontrol Strategy for Increasing Resistance to Spring Dead Spot in Bermudagrass

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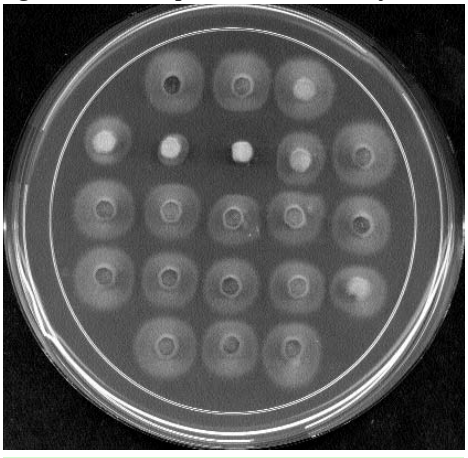
Objectives:

1. Isolate and identify additional antagonistic endophytes or rhizosphere-competent bacteria with antagonistic properties against *Ophiosphaerella herpotricha*.
2. Develop a successful biotization strategy to introduce stable colonizing endophytes into bermudagrass tissues.
3. Screen the biotized endophyte-plant associations selected in objective 2 in the field for effectiveness in inhibiting the development of SDS.

Start Date: 2003
Project Duration: three years
Total Funding: $60,000

Spring dead spot (SDS) is a major disease that afflicts bermudagrass throughout the northern range of its adaptation. The disease is caused by three root-rotting fungi: *Ophiosphaerella herpotricha*, *O. korrae*, and *O. narmari* all of which are found within the United States. Symptoms appear in the spring as unsightly patches of dead and dying turf anywhere from and six inches to three feet in diameter. Modified cultural practices and improved plant resistance help, but do not prevent disease occurrence.

We found a *Bacillus subtilis* strain that strongly inhibited the growth of *O. herpotricha* in the laboratory. We have isolated, identified, and characterized some of the active ingredients, which includes several antifungal peptides. We plan to use this agent to try to improve control measures. One problem with conventional biocontrol is that the introduced agent must compete with an already established microflora. Biotization may increase the competitive ability of the introduced agents by giving it a leg up on the competition. This is done by inoculating the seedling with the biocontrol agent prior to planting in a soil medium. The theory behind the biotization strategy is that the first microorganisms to colonize the rhizosphere will have the competitive advantage over the latecomers.



Oklahoma State University research found a Bacillus subtilis *strain that strongly inhibited the growth of* O. herpotricha *in the laboratory. They have isolated, identified and characterized some of the active ingredients, which includes several antifungal peptides.*

Another improvement in biocontrol may involve antagonistic endophytes. Endophytes are microorganisms that are already well adapted to living within plant tissues. We plan to isolate antagonistic bermudagrass endophytic bacteria and biotize them at high concentrations into the bermudagrass seedlings prior to planting. We think the combination of biotization with antagonistic endophytes will produce an effective strategy to supplement improvements in plant resistance and cultural practices.

We have isolated over a hundred antifungal endophytes with antagonistic activity against the causal agent of SDS, *Ophiosphaerella herpotricha*. Putative identification of these endophytes using a partial fragment of the 16S ribosomal gene classified them into a diverse array of 20 genera, two of which have never before shown to contain endophytic species. In addition, we completed a more quantitative evaluation of antifungal activity from each isolate.

We are in the process of obtaining the 16S-intergenic sequence from the most potent of all antifungal isolates. We have successfully purified and identified two antifungal peptides from the inoculation media of one potent antifungal bacterium (AF1). In addition, we were successful in purifying some additional unknown peptides with antifungal activity and are in the process of performing structural determination on these compounds. In addition, we sequenced and analyzed the entire 16S and intergenic region for AF1.

Researchers believe that by inoculating bermudagrass seedlings with Bacilus subtilis, *this may make the bermudagrass host resistant to infection by the SDS pathogens.*

We are currently developing a biotization strategy for introducing the endophytic bacteria into seeded bermudagrass. Using our *in vitro* system, we have successfully infected bermudagrass with *O. herpotricha* and are implementing procedures to enhance antifungal activity.

This last summer we field inoculated plots infected with *O. herpotricha* with AF1. First years results will be available in the spring of 2005.

Summary Points

- Established phylogenetic relationship among antifungal endophytes.
- Developing a biotization strategy and screening system for effectiveness in inhibiting *O. herpotricha*.
- Began field testing AF1.
- Purified additional antifungal compounds and obtained additional phylogenetic information from AF1.