Transformation of Bermudagrass for Improved Fungal Resistance

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Objectives:

1. Isolate, identify, and characterize chitinases and glucanases and their genes that have high activity against Spring Dead Spot (SDS).
2. Develop an efficient protocol to transform (genetically engineer) bermudagrass.
3. Transform and characterize bermudagrass with the antifungal chitinase and/or glucanase genes directed against the SDS casual organism.

Start Date: 1998
Project Duration: 5 years
Total Funding: $125,000

Spring dead spot (SDS) causes significant economic damage to bermudagrass in the Southeastern United States. The causal agents are *Ophiosphaerella herpoiricha, 0. korrea* and *0. narmari*. They are most active in the early fall and spring when temperatures and moisture favor fungal growth over that of bermudagrass.

Our initial objectives were to isolate antifungal chitinases and glucanases and to develop an efficient transformation system for bermudagrass. However, attempts to identify chitinases with high antifungal activity were unsuccessful. Chitinases from potato, spinach, bean, and bermudagrass showed no activity against *0. herpotricha*. Experiments to transform bermudagrass produced 971 putative transformants, but after a thorough evaluation at the biochemical and molecular levels, we concluded that none of these plants were transgenic.

Modifications of the original objectives were necessary due to the inability to find antifungal chitinases and to efficiently transform bermudagrass. At about the same time we were investigating the antimicrobial properties of chitinases we discovered a microorganism that showed dramatic growth inhibition against the fungus. Comparison of the bacterial 16S ribosomal RNA sequence with those in the NCBI and Michigan State Database revealed the bacterium to most closely resemble the *Bacillus subtilis* species.

Many members of this species are known to be well adapted to the soil medium, rhizosphere and endorhizosphere and several members of this species have been developed as commercial biocontrol agents. Antibiotics excreted into the growth medium were identified as surfactin, iturin and a novel bacitracin like compound. To test for biocontrol efficacy we have inoculated field plots and plan to initiate evaluations in the fall of 2002.

Further experiments resulted in the isolation of well-adapted endophytes with antifungal activity that may allow for the development of an endophyte biocontrol system.

The new cold-hardy, seeded bermudagrasses from Oklahoma State University (below) have superior spring dead spot resistance than older seeded cultivars (above).

We have transformed another 300 plants via particle bombardment with a GFP marker. Unfortunately, no transgenics were recoverable due to the high level of native fluorescence in bermudagrass tissues. In addition, we are currently developing a tissue culture system that regenerates from dedifferentiated nodal tissues of bermudagrass in order to improve the efficiency and capacity of our tissue culture/transformation process.

A better understanding of the basic biology behind bermudagrass infection by fungal pathogens will spur new and novel strategies to improve resistance to pathogen attack. At OSU we are using a genomic approach to investigate the resistance mechanism to SDS in bermudagrass. Several libraries containing genes were created using suppressive subtractive Over 800 clones have been sequenced from these libraries and 98 sequences have been processed through PipeOnline, a bioinformatics program (OSU bioinformatics group). These sequences were most similar to sequences involved in cell maintenance and development (62%), high molecular weight defense genes (15%), unknowns (15%), stress response and cell death (5%), and low molecular weight defense genes (3%). Processed EST representing the first bermudagrass sequences were deposited in the GenBank dbEST.

Next year we will finish sequencing clones and in collaboration with the Samuel Roberts Noble Foundation conduct microarray analyses to evaluate differential expression under a variety of conditions involving resistance and susceptibility to fungal infection.

Summary Points

- No chitinases were discovered with antifungal activity against the causal agents of SDS, *0. herpotricha.*
- Over 971 putative transgenics were shown not be transgenic when screened using molecular and biochemical techniques.
- A potential biocontrol, *Bacillus subtilis* species, was discovered and shown to produce three stable antibiotics against *0. herpotricha.* An additional 25 anti-*O. herpotricha* microorganisms were identified and characterized.
- Genomic analysis for resistance to SDS was initiated with many genomic fragments sequenced, characterized and submitted to the GenBank dbEST database.