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.

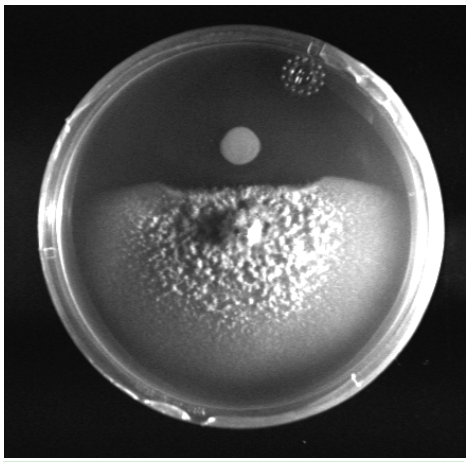
Start Date: 1998
Project Duration: 5 years
Total Funding: $125,000

Attempts to identify chitinases and glucanases with high activity were unsuccessful. Chitinases from potato, spinach, bean, and bermudagrass showed no activity against *O. herpotricha* using our standard growth inhibition assay. Modifications of the original objectives were necessary due to the inability to find antifungal chitinases. 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. Our plan was to develop this bacterium as a biocontrol agent against spring dead spot.

Since many previous biocontrol agents have proven to be ineffective against plant diseases we decided to search for additional microbial antagonists. The search included a class of bacteria that are well adapted to living symbiotically within the hosts tissues known as endophytes. Unlike some other potential biocontrol agents, these endophytes would be well adapted and presumably competitive within the host tissues.

We isolated over 1,500 endophytic isolates from bermudagrass crown tissues after an exhaustive surface sterilization to form the backbone of our endophytic library. We collected endophytes from both infected and non-infected varieties of resistant Midlawn and susceptible Tifgreen. Of the 1532 isolates, 129 were sequenced and putatively identified by their 16S ribosomal gene sequence.

Bermudagrass endophytes comprise at least 19 genera with 22 distinct species. The most abundant genus were *Microbacterium* followed by *Stenotrophomonas* and *Pseudomonas*. Many of these plant genera were previously shown to be endophytic in other plant species.



Inhibition of O. herpotricha *associated with* Bacillus subtilis *biocontrol agent on potato dextrose agar plates*

The endophytes were screened for inhibitory activity against the *O. herpotricha.* The antifungal screen of 808 endophytes revealed 131 isolates with moderate levels of antagonistic activity or 16% of those tested. Those that showed the highest level of endophytic activity were from the genus *Pseudomonas* and *Stenotrophomonas*.

Experiments to transform bermudagrass using the gene-gun approach were conducted over the last four years. These experiments produced 971 putative transformants. After thorough evaluation of these materials at the biochemical and molecular levels, we concluded that none of these plants were transgenic.

The objective of our current and ongoing research is to identify bermudagrass genes conferring resistance to SDS. Differentially expressed (both up and down regulated) gene transcripts were selected from two sets of samples of seeded bermudagrass cultivars (2n=4x=36) [resistant cultivar "Yukon" vs. susceptible cultivar "Jackpot"; infected tissue vs. non-infected tissue] by Suppression Subtractive Hybridization (SSH) to create a normalized cDNA library of the mRNA population. Four SSH libraries that contain 834 fungal-induced gene transcripts were created. The average insert size was 400 base pairs. All of these clones were sequenced.

A unique set of 661 sequences have been identified, annotated, and deposited in dbEST of GenBank. Putative function of the differentially expressed genes was determined by database searches (GenBank). Six categories of genes involved in host-pathogen interactions were identified: 1) anti-microbial, 2) general stress response, 3) low molecular weight defense signals, 4) high molecular weight signal regulation, 5) cell maintenance, and 6) development.

Sequence data indicated that disease resistance was associated with a complex plant defense response which involved an integrated set of genes. These ESTs have been used to print cDNA microarrays to establish expression profiles associated with resistance to SDS. One hundred and twenty genes were identified as being differentially expressed in response to fungal infection.

Summary Points

- Antifungal *Bacillus subtilis* species was characterized with respect to the antagonistic principle.
- Endophytes from bermudagrass isolated and identified, including 131 with moderate level of antagonism.
- Suppression Substractive Hybridization (SSH) libraries were constructed and characterized that contained 661 unique gene fragments.
- Microarray analysis of *O. herpotricha* induced genes was performed identifying 120 differentially expressed genes.