Control of Bentgrass Pathogenic Fungi Dollar Spot, Brown Patch and Pythium Blight Using Chitinase

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Goals:

- *Express our cloned chitinase gene in E. coli, and purify and collect chitinase protein.*
- *Identify the level of chitinase required to control three major turfgrass pathogens.*
- *Transform bentgrass with plasmids containing the chitinase gene.*
- *Evaluate the transgenic plants for resistance to major turfgrass pathogenic fungi.*

Cooperators:

Dr. Mariam Sticklen
Dr. Bruce Branham

Dollar spot *(Sclerotinia homeocarpa)*, brown patch *(Rhizoctonia solani)* and pythium blight *(Pythium aphanidermatum)* are major pathogenic diseases of turfgrass. All of these pathogens contain chitin in their cell walls, and therefore may be susceptible to chitinases. Also, all of these pathogens contain proteinases which are essential for the survival of the pathogenic fungi.

The research project has cloned and characterized a full length chitinase gene which contains the necessary chitin-binding domain. Several plasmids were constructed that contain a potato proteinase inhibitor II controlled by different (monocot-specific, wound-inducible, and constitutive) promoters.

During the first year of the project, the chitinase gene was successfully expressed in *E. coli.* The expression of this plant gene in *E. coli* was confirmed by extracting a chitinase-containing slurry. Dr. Vargas's laboratory examined the effect of the recombinant chitinase on turfgrass pathogenic fungus, *R. solani.* Due to the hydrophobic (water insoluble) nature of the expressed chitinase, the bioassay did not provide accurate results. Work is in progress to remove the hydrophobic portion of this gene, and express the modified gene in *E. coli* again.

The chitinase gene also was successfully expressed, in a homozygous state, in second generation tobacco plants. Tobacco is relatively quick and easy to transform. This transformation provides information about the plant's ability to produce active chitinase from this gene. If tobacco can successfully

produce the chitinase with antifungal activity, it would indicate that bentgrass plants will likely do the same. Dr. Vargas's laboratory is testing these plants against *R. solani.* Work is in progress to express this chitinase gene in creeping bentgrass.

During the first year of the project, the potato proteinase inhibitor II and the *bar* (bialaphos - Ignite or Finale™ herbicide resistance) genes were successfully expressed in creeping bentgrass. To date, thirty seven independent groups of transgenic creeping bentgrass (with different sites of gene insertion) have been identified. Professor Donald Penner, the MSU herbicide physiologist, has confirmed the degree of resistance of these transgenic plants to bialaphos. Several of these independent transgenic plants have been transferred to the field. Further analysis against both Finale™ herbicide and turfgrass pathogenic fungi will be performed on these plants during Spring and Summer of 1996.