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.

More than one thousand creeping bentgrass plants have been putatively transformed for herbicide and insect resistance with the *bar* and *pinII* genes. They exhibit resistance to Ignite herbicide (1.2% commercial product containing 200g/L active ingredient of glufosinate ammonium) foliar spray. Molecular analysis of these plants is in progress.

A chitinase gene cloned from elm trees has been successfully manipulated to express in *E. coli*. The *E. coli* cells with the gene produced the GST-chitinase fusion protein. Attempts to purify the GST-chitinase fusion protein were not successful. Therefore, experiments were set up to collect fractions of the bacterial extract at several steps during the purification process. These experiments resulted in discovering that the GST-chitinase fusion protein was not soluble in the buffer used.

In an effort to remedy this situation, two steps have been taken. The first step is to remove the signal peptide currently located at the amino terminal of the protein. This amino acid sequence is 16 amino acids in length and is highly hydrophobic, which may be contributing to the insolubility of the GST-chitinase fusion protein. A PCR primer has been designed and will be used to produce a PCR product containing the chitinase gene without the hydrophobic signal peptide. This PCR product will be cloned into a bacterial expression vector. The construct will be used to transform *E. coli* and will be induced to determine if they contain soluble chitinase with antifungal activity.

The second step underway is transformation of tobacco with the chitinase gene isolated from elm. Tobacco is relatively quick and easy to transform via *Agrobacterium*. This successful transformation

will give us information about the plant's ability to produce active chitinase from this gene. If tobacco can successfully produce elm chitinase with antifungal activity, it would indicate that bentgrass plants will likely do the same. At that point it is expected that no further manipulation of the elm chitinase gene will be necessary before the transformation of bentgrass plants. Upon successful transformation of tobacco, leaf extracts will be used in bioassays to test for antifungal activity.

Antifungal bioassays have been attempted. However, due to the insolubility of the GST-chitinase fusion protein produced in bacteria, no activity has yet been observed.