THE ROLE OF HYDROLYTIC ENZYMES IN THE BIOCONTROL ACTIVITY OF *TRICHODERMA VIRENS*

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Abstract

Strains of the filamentous fungus *Trichoderma virens* are effective biocontrol agents against a range of plant pathogens. The mechanisms that have been proposed to describe how mycoparasitic strains antagonize their hosts include the activity of cell-wall degrading enzymes, antibiosis, induction of host defense compounds, and competition for nutrients and/or infection sites. These mechanisms may work in concert such that synergistic interactions develop. Understanding the components of these mechanisms will greatly assist in further developing reliable and effective biocontrol agents. We have adopted a molecular approach in an effort to determine the role of several cell-wall degrading enzymes and membrane-altering compounds (antibiotics). The specific objectives of this research were to: 1) identify, isolate and clone genes that have the potential to enhance biocontrol activity, 2) determine the role of these genes in the biocontrol activity of *T. virens*, 3) construct strains of *T. virens* that over-express selected genes for enhanced biocontrol activity and 4) produce transgenic plants that express selected fungal genes involved in mycoparasitism.

The genes of interest are being identified and isolated by two approaches:
1) PCR-based cloning using degenerative primers of conserved regions from selected genes in other organisms and 2) screening differentially expressed cDNA libraries. Using the PCR-based approaches, we have cloned 7 chitinases, 3 glucanases and one proteinase and a peptide synthetase that encodes a peptaibol antibiotic. Strains of *T. virens* were constructed such that an endochitinase *(cht42)* or a proteinase (*prov1)* were over-expressed or disrupted. The resulting transformants were stable and similar to the wild-type strain with respect to growth rate, sporulation and antibiotic production. In the case of *cht42*, the biocontrol activity of the disrupted and the over-expression strains were significantly decreased and enhanced, respectively against cotton seedling disease incited by *Rhizoctonia solani* when compared to the wild-type strain. *In vitro* tests demonstrated greater inhibition of growth of *R. solani* by the over-expression than the disrupted strain or the wild-type parent. Based on the results of single gene over-expression, we have begun to transform *T. virens* with gene combinations (eg. *cht42* plus *prov1* or *cht42* plus a gene encoding a β-1,3 glucanase). These transformants will be assayed to determine if a synergistic effect can be obtained by over-expressing enzyme combinations. The differential screening assay has identified 95 clones that are potentially unique to the interaction of *T. virens* and *R. solani.* Full length copies of the genes for unique clones identified by sequence analysis will be obtained by screening a genomic cosmid library of *T. virens.* These approaches allow the identification of genes involved in mycoparasitic interactions that may used to enhance biocontrol activity of biocontrol agents or increase the resistance in transgenic plants.

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