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Cloning of a chitinase from *Trichoderma viride* for the development of transgenic plants resistant to fungal diseases

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Phytopathogenic fungi are responsible for 70% of the harvest failures worldwide. Although there are several methods for managing fungal diseases, including the use of fungicides etc., many of these have several disadvantages. This investigation was focused on increasing the ability of plants to resist pathogenic fungi by transforming plants with a mycolytic enzyme, endochitinase (42 kDa), an extracellular enzyme, isolated from the fungus *Trichoderma viride,* which is the most popular biocontrol agent used extensively against pathogenic fungi.

Fungal RNA was purified from two-day old mycelia grown on a media containing chitin using Tri reagent, a mixture of guanidium thiocyanate and phenol. The extracted RNA was reversed transcribed using oligo (dT). The cDNA was then amplified using TVC-FP-NSP and TVC-RP-NSP primers, flanking the coding region of chitinase. The amplified product was TOPO cloned in pCR2.1 vector (Invitrogen) and a putative clone with an insert of 1275 bp was sequenced and analyzed.

A search of the non redundant nucleotide database at National Center for Biotechnology Information (NCBI) revealed a high degree of homology (98%) to a previously cloned endochitinase of *T. viride.* The deduced amino acid sequence was aligned with the above previously cloned amino acid sequence of the *T. viride* endochitinase gene. A high degree of homology (99%) was observed with few changes at some positions. These include, positions 71 (Proline to Serine), 78 (Aspartate to Glycine), 100 (Asparagine to Serine), 111 (Asparagine to Lysine) and 255 (Asparagine to Lysine). None of these changes involved the active site residues, the consensus regions of the catalytic domain (SxGG) or the substrate binding domain (DxxDxDxE) common to family 18 fungal chitinases.

Currently, work is underway to clone the isolated sequence in the expression vectors pRTL2- GUS and pCAMBIA1303 for *Agrobacterium* mediated transfer initially to rice.

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