

## THE MOLECULES THAT ACTIVATE ANTAGONIST FUNGI USED AS BIOPESTICIDES

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Fungi of the genus *Trichoderma* are biological control agents commonly applied against a variety of plant diseases. Pathogens and plants release molecules that are detected by antagonists and induce the expression of biocontrol genes. We used two *Trichoderma* mutants reporter gene systems based on the GFP and a gene encoding for a glucose oxidase under the control of biocontrol-related promoters to select low molecular weight compounds acting as “biocontrol inducers”.

Various compounds capable of producing these inducers were tested singly and in combination: purified *Trichoderma* enzymes (endochitinase, exochitinase, chitobiosidase and glucanase); culture filtrates (CFs) containing extracellular enzymes coming from *T. atroviride* P1 (wild-type and knock out mutants), *T. harzianum* and *T. resei*; CFs of the pathogens *Botrytis*, *Pythium* and *Rhizoctonia*; colloidal crab shell chitin; plant extracts from cucumber and tomato leaves, stems and roots. CFs from chitinase knock-out mutants and cell walls from Oomycete fungi were the less active. The compounds of MW less than 3kDa obtained from the host CW digestion were found to strongly activate *Trichoderma* gene expression as well as stimulate its mycelial growth and spore germination. HPLC-purified fungal host-derived inducers stimulated the production by *Trichoderma* of endochitinase and exochitinase even under repressing conditions in presence of glucose. These compounds assayed *in vivo* were also able to reduce disease symptoms induced by *B. cinerea* on bean leaves enhancing biocontrol effect of P1. Finally, purified inducers added to *T. atroviride* cultures stimulated the production of antibiotics that inhibited *Botrytis* and *Alternaria* spore germination. Mass spectrometry analysis (EMI-MS) of the inducers indicated the presence of hexose oligomers like cellobiose, whereas MS/MS-analysis by selective fragmentation of peaks in the spectrum demonstrated the presence of at least three distinct biologically active compounds.