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Enhancement Of Plant Disease Resistance By The Biocontrol Agent *T. asperellum*

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Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly, ecological approach to overcome the problems caused by standard chemical methods of plant protection.

Mycoparasitism

Trichoderma spp. are free-living fungi that are very common in soil and root ecosystems. They are effective biocontrol agents of several plant pathogens and their interaction with soilborn pathogenic fungi is an excellent example for mycoparasitism. Mycoparasitism appears to be a complex process, made up from several successive steps. Upon contact with the host, *Trichoderma* coils around or grows along the host hyphae and forms hook-like structures that aid in penetrating the host's cell wall. In *Trichoderma*, this reaction has been found to be rather specific, and lectin-carbohydrate interactions were assumed to mediate the attachment and recognition between *Trichoderma* and soil-born plant pathogenic fungi. As a result of the recognition process, a group of extracellular fungal cell wall degrading enzymes is produced, mostly chitin-degrading enzymes, named chitinases. Chitin is one of the most abundant polymers in nature and occurs as a structural polymer in most fungi and insects. Chitinases are therefore under extensive study, in an attempt to elucidate their role in biological control. We isolated and characterized the regulation of three chitinase genes from *Trichoderma asperellum* and demonstrated their direct involvement in the mycoparasitic process. The presence of highly homologous versions of these genes in different *Trichoderma* strains suggest the importance of these gene products. Their presence appears to be of selective advantage for antagonistic performance and improvement of plant disease resistance.

Plant induced disease resistance

Recent discoveries demonstrate that *Trichoderma spp.* are opportunistic avirulent plant symbionts. At least some strains establish robust and long-lasting colonization of root surfaces and penetrate into the epidermis and a few cells below this level. They produce or release a variety of compounds that

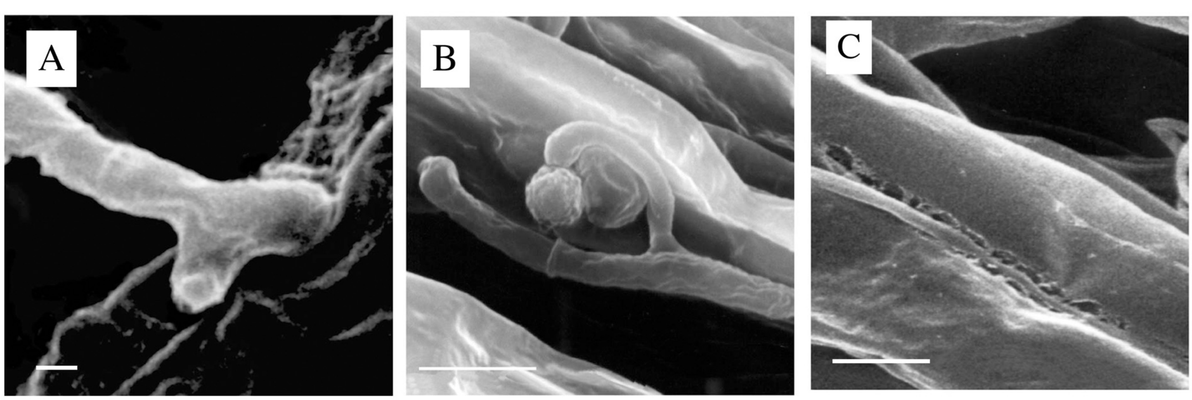

Fig. 1 *Interactions of Trichoderma asperellum with cucumber roots*
A:Appressoria-like structures B: Coils around root hairs C: Root cortical cells penetration

induce localized and systemic resistance responses and cause substantial changes in the plant proteome and metabolism.

Using an aseptic hydroponics growth system we have conclusive evidence for the induction of a systemic response against angular leaf spot of cucumber-*Pseudomonas syringae* pv. *lachrymans* (Psl), following application of *T. asperellum* to the root system. Disease symptoms were reduced by as much as 80%. As shown by electron microscopy, bacterial cell proliferation in these plants was halted. The protection afforded by the biocontrol agent was associated with the accumulation of mRNA of two defense genes that form part of the phytoalexins synthesis pathway. This was further supported by the accumulation of secondary metabolites of a phenolic nature showing up to six fold increase in inhibition capacity of bacterial growth in vitro. The bulk of the antimicrobial activity was found in the acid-hydrolyzed extract containing the phenolics in their aglycone form. HPLC analysis of phenolic compounds showed a marked change in their profile, in the challenged pre-elicited plants relative to challenged controls.

Moreover, challenge of *Trichoderma* induced plants with the leaf pathogen *Pseudomonas syringae* pv. *lacrymans* results in higher systemic expression of some plant defense related genes, indicating that the *Trichoderma*–plant interaction induces a 'potentiated' state in the plant. This enables higher resistance to subsequent pathogen infections. Combining gene expression analysis, measurements of signal molecules levels and use of specific hormone inhibitors we could demonstrate that the JA/ethylene signaling pathway is involved in the systemic response induced by *Trichoderma*.

Trichoderma and cucumber seedlings are used in our laboratory also as a model to study the modulation of *Trichoderma* gene expression during the first stages of plant root infection. This preliminary study reveals that molecules related to phytopathogenicity, but also known as elicitors of plant defense responses, are transiently activated *in planta*, enabling at the same time intracellular colonization and induction of systemic defense responses pathways.

Our research clearly shows that in addition to the ability of *Trichoderma spp*. to directly attack or inhibit growth of plant pathogens, these fungi can also induce systemic and localized resistance to a variety of plant pathogens. These new findings are dramatically changing our knowledge of the mechanisms of action and uses of this biocontrol agent.

Selected Publications

Yedidia, I., Benhamou, N., and Chet, I. (1999) Induction of defense responses in cucumber plants (*Cucumis sativus L*.) by the biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microbiol. 65:1061-1070.

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Harman, G.E., Howell, C.R., Viterbo, A, Chet, I. and Lorito, M. (2004) *Trichoderma spp.*—Opportunistic avirulent plant symbionts. Nature Microbiol. Rev. 2: 43-56.

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