Boosting plant defence by beneficial soil microorganisms

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Plants in their environment face potential deleterious organisms such as fungi, bacteria, viruses, nematodes, etc. Many of them are able to cause plant diseases, responsible of important losses in crop production worldwide. But often the outcome of these interactions is not disease, since plants have developed multiple mechanisms to protect themselves against pathogens attack. Moreover, beneficial microorganisms are common in the soil, improving plant growth and reducing the effects of deleterious organisms. While chemical control of plant diseases is usually expensive and may have a negative impact on the environment and on public health, the use of microorganisms to control plant pathogens, known as biological control, is accepted as a durable and environmentally friendly alternative in plant disease management.

Several modes of action have been described in biological control. Direct effects of the biocontrol agent over the pathogen include inhibition by antimicrobial compounds (antibiosis), competition for colonization sites and nutrients, degradation of pathogenicity factors and parasitism. Indirect mechanisms include improvement of plant nutrition and damage compensation, changes in the root system anatomy, microbial changes in the rhizosphere and activation of plant defence mechanisms, leading to enhanced plant resistance. It is common that an effective biocontrol agent acts through the combination of different mechanisms (Whipps, 2001).

For example, the filamentous fungi *Trichoderma spp.* have been widely studied for their effectiveness in controlling a broad range of phytopathogenic fungi such as *Rhizoctonia solani*, *Pythium ultimum* and *Botrytis cinerea*. The mechanisms involved in this protective effect are mainly direct, through antibiosis and parasitism. *Thichoderma* grows around the fungal pathogen (Fig. 1) and releases toxic compounds and a battery of lytic enzymes, mainly chitinases, glucanases and proteases. These proteins facilitate *Trichoderma* penetration into the host and the utilization of the host components for nutrition. The implication of lytic enzymes in biocontrol has been confirmed in overproducing mutants (Mendoza-Mendoza et al., 2003; Pozo et al., 2003), and the expression of some of these enzymes in transgenic plants highly increased their resistance to different pathogens (Emani et al., 2003).

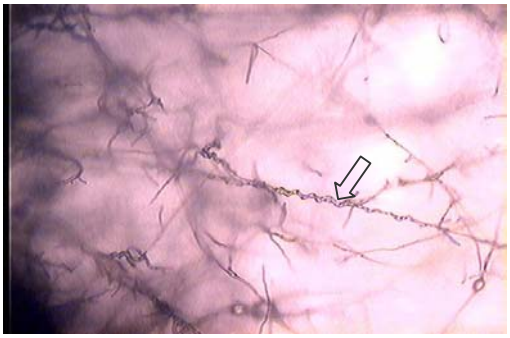

Figure 1. The biocontrol fungus *Trichoderma virens* grows around hyphae of the pathogenic fungus *Rhizoctonia solani.* While growing around its host, or "coiling", *Trichoderma* secretes different lytic enzymes able to degrade fungal cell walls, allowing the penetration and parasitation of the host.

Arbuscular mycorrhizal fungi (AMF), which form symbiotic associations with root systems of almost all plants, also reduce root diseases caused by several soil-borne pathogens, mainly through indirect mechanisms. The AMF penetrates the root system (Fig. 2A), improving plant nutrition and growth and altering the anatomy and architecture of the root system. These changes, together with the activation of the plant defence mechanisms, seem to be responsible for the reduction of the disease (reviewed in Azcón-Aguilar et al., 2002; Pozo et al., 2002a). For example, colonization of tomato roots by *Glomus mosseae* reduce disease development in plants infected with *Phytophthora parasitica* (Fig. 2B), and the involvement of plant defence mechanisms has been pointed out (Pozo et al., 1996; Cordier et al., 1998; Pozo et al., 1998; Pozo et al., 1999; Pozo et al., 2002b).

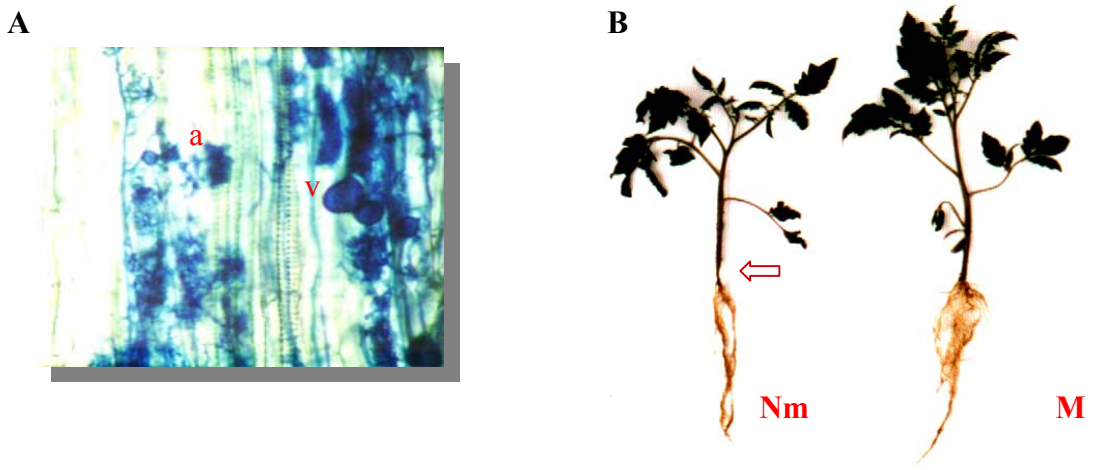

Figure 2. A. Tomato roots colonized by the mycorrhizal fungus *Glomus mosseae* were stained with trypan blue to detect fungal structures. The fungus develops inside the root cortical cells forming vesicles (v) and specialized structures called arbuscules (a). **B.** After *Phytophthora parasitica* infection, non-mycorrhizal tomato plants (Nm) showed strangulated collar (arrow), extensive necrotic areas in the root system and a decrease in the root and shoot biomass. In contrast, plants colonized by *G. mosseae* (M) showed no symptoms in the collar, very limited necrosis in the roots and normal biomass development.

But one of the most studied biocontrol organisms are bacteria from the genus *Pseudomonas*. They constitute an excellent example of combination of multiple mechanisms for effective biocontrol (reviewed in Van Loon et al., 1998). *Pseudomonas spp.* produce several metabolites with antimicrobial activity towards other bacteria and fungi. They also produce siderophores that will restrict pathogen growth by limiting the iron available in the soil. Remarkably some strains are also able to trigger an induced resistance that enhances the defensive capacity of the plant to a subsequent pathogen attack. This effect is not localized at the colonization site in the roots, but systemic, conferring the plant a better protection not only against a broad range of soil pathogens, but also to foliar ones (Fig. 3). This phenomenon is known as rhizobacteria-mediated Induced Systemic Resistance or ISR (Van Loon et al., 1998). Interestingly, no major changes in gene expression in the plant have been related to the ISR state. Instead, induced plants show a faster or greater activation of defence responses after infection with a challenging pathogen -a phenomenon called “potentiation” or “priming”- (Conrath et al., 2002).

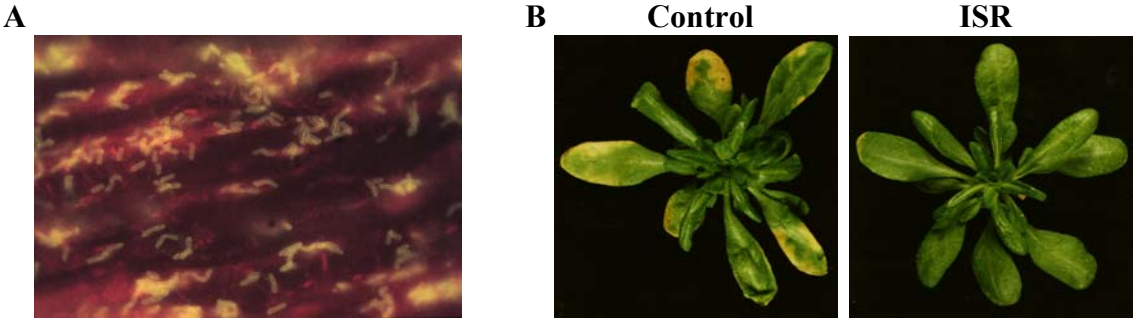

Figure 3. A. *Pseudomonas fluorescens* WCS417r bacteria on the surface of a plant root visualized by green fluorescence labelled antibodies. **B.** Treatment of *Arabidopsis* roots with *P. fluorescens* WCS417 promotes Induced Systemic Resistance (ISR) evidenced in the picture by the reduction in disease symptoms after inoculation with the bacterial leaf pathogen *Pseudomonas syringae* pv. *tomato* compared to controls (reproduced from Pieterse and Van Loon, 1999).

Understanding the genetic control of the plant defence-related processes underlying ISR is a key point in biocontrol research. The complexity of these mechanisms, regulated by multiple genes, requires the use of a well-defined biosystem and high-throughput techniques for the analysis of gene expression, such as microarrays. The use of the model plant *Arabidopsis thaliana* has greatly contributed to the progress in this area due to the availability of mutant lines in different signal pathways and the sequencing of its genome in full. Indeed, great advances in our knowledge in plant defence reactions have been achieved in recent years. It is now known that plant inducible defence pathways are regulated through a complex network of signalling cascades that involve three main molecules: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), enabling the plant to fine-tune its resistance reaction depending on the micro-organism encountered (Pieterse and Van Loon, 1999). The Phytopathology group in Utrecht has shown that ISR acts through the JA and ET signalling pathways, but it is independent on SA (Pieterse et al., 1996; Pieterse et al., 1998). However, analysis of local and systemic levels of JA and ET showed no changes in their production. This result suggested that ISR is based on an increased sensitivity to these plants hormones, and not on changes in their production (reviewed in Pieterse et al., 2002). To confirm this hypothesis, we are investigating if ISR-expressing plants are primed to react faster or more strongly to JA or ET produced after pathogen infection. With this aim, the induction of defence-related genes by different concentrations of ET and JA was compared at several times in *Arabidopsis* plants treated or not with the ISR inducing *Pseudomonas fluorescens* WCS417 bacteria. As an example, fig. 4A shows the quicker and higher increase in the expression of *LOX2*, a gene involved in the synthesis of JA, in ISR-expressing plants compared to the controls after treatment with methyl jasmonate. In another experiment (Fig. 4B), ET application at different concentrations resulted in higher transcript levels of the ethylene biosynthesis gene *ACO* in ISR-expressing plants compared with controls. These results indicate that priming of specific sets of JA- and ET-responsive genes is indeed associated to ISR. We hypothesize that priming of pathogen-induced genes allows the plant to react more effectively to the invader encountered, which might explain the broad-spectrum action of rhizobacteria-mediated ISR. To determine the full set of genes involved in the process, we are at the moment analyzing the expression of thousands of genes in response to ET, JA and/or pathogen attack in ISR-expressing or control plants by microarray screenings.

Figure 4. Priming of JA-induced *LOX2* and *ET*-induced *ACO* gene expression in ISR-expressing *Arabidopsis* plants after induction by *P. fluorescens* WCS417 (ISR). **A.** Expression of *LOX2*, involved in jasmonate signalling, 0, 1, 3, 6 and 12 hours after treatment with 50 µM methyl jasmonate. **B.** Expression of *ACO*, an enzyme involved in ethylene signalling, after 6 hours of treatment with different ethylene concentrations (0, 0.1, 1 and 10 ppm). Control, non-induced *Arabidopsis* plants.

Although important advances have been achieved lately in our knowledge of plant defence mechanisms and its induction, many aspects remain unclear. Understanding the mechanisms by which plants perceive and respond to micro-organisms that stimulate their natural defences will provide more insight into how plants can be helped to defend themselves against pathogen attack and constitutes a very promising research area.

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