Microbiology Papers in Press. Published September 13, 2012 as doi:10.1099/mic.0.061457-0

Pythium oligandrum: an example of opportunistic success

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Pythium oligandrum, a non-pathogenic soil-inhabiting oomycete, colonizes the root ecosystem of many crop species. Whereas most members in the genus *Pythium* are plant pathogens, *P. oligandrum* distinguishes itself from the pathogenic species by its ability to protect plants from biotic stresses in addition to promoting plant growth. The success of *P. oligandrum* at controlling soilborne pathogens is partly associated with direct antagonism mediated by mycoparasitism and antimicrobial compounds. Interestingly, *P. oligandrum* has evolved with specific mechanisms to attack its prey even when these belong to closely related species. Of particular relevance is the question of how *P. oligandrum* distinguishes between self- and non-self cell wall degradation during the mycoparasitic process of pathogenic oomycete species. The ability of *P. oligandrum* to enter and colonize the root system before rapidly degenerating is one of the most striking features that differentiate it from all other known biocontrol fungal agents. In spite of this atypical behaviour, *P. oligandrum* sensitizes the plant to defend itself through the production of at least two types of microbe-associated molecular patterns, including oligandrin and cell wall protein fractions, which appear to be closely involved in the early events preceding activation of the jasmonic acid- and ethylene-dependent signalling pathways and subsequent localized and systemic induced resistance. The aim of this Review is to highlight the expanding knowledge of the mechanisms by which *P. oligandrum*

provides beneficial effects to plants and to explore the potential use of this oomycete or its metabolites as new disease management strategies.

Introduction

In a large number of agricultural ecosystems, crop production is severely hampered by soilborne pathogen-elicited diseases that cause heavy economic losses (van West *et al.*, 2003). In spite of significant advances in crop protection (i.e. crop rotation, use of chemical pesticides and breeding of resistant crop varieties), soilborne plant pathogens still significantly reduce yield and quality in diverse economically important crops. These pathogens are particularly challenging because they often survive in soil through resilient survival structures. In the past few decades, the expanding movement toward more environmentally friendly agricultural practices has accelerated the search for alternative strategies that could provide safe and reliable means to combat root diseases (Alabouvette *et al.*, 2006). Among the proposed approaches, the exploration of the mechanisms by which naturally occurring beneficial micro-organisms can suppress disease incidence or severity has attracted much attention in relation to their potential at fighting root pathogens and modulating the plant immunity (Benhamou *et al.*, 1997, 1999; Harman *et al.*, 2012; Validov *et al.*, 2011; Yedidia et *al.*, 1999).

While the beneficial effects of rhizobacteria and true fungi such as species in the genus *Trichoderma* have been abundantly explored (Berg, 2009; Lorito *et al.*, 2010), the possibility that selected members of the oomycetes may contribute to protecting plants from infection has often been ignored by plant pathologists who long considered that oomycetes were essentially virulent pathogens (Blair *et al.*, 2008; Latijnhouwers *et al.*, 2003). In the past few years, however, the field of beneficial plant–oomycete interactions has started to be the focus of interest and it is anticipated that scientists from both fields will work together to provide an integrated view of the molecular mechanisms that evolved in pathogenic and beneficial plant–oomycete interactions. Within the oomycetes, the genus *Pythium* (about 130 species) occupies a large variety of terrestrial and aquatic ecological niches (Lévesque & de Cock, 2004). Some members of this genus are active saprophytes, others are pathogens on an array of organisms including algae, fish and insects (Van der Plaats-Niterink, 1981) while the most economically important members are plant pathogens with a broad host range (Thines & Kamoun, 2010). That beneficial relationships between plants and some *Pythium* species may occur in nature has long been postulated (Drechsler, 1943), but it is only since 2000 that the intimate relationships established between plants and beneficial *Pythium* species has been deeply explored (Mohamed *et al.*, 2007; Picard *et al.*, 2000b; Takenaka *et al.*, 2008). Among the few mycoparasitic *Pythium* species so far identified, *Pythium oligandrum* Drechsler, a soil inhabitant with a worldwide distribution, is undoubtedly considered to be the most promising biocontrol oomycete for use in agriculture (Rey *et al.*, 2008).

The beneficial effects of *P. oligandrum* on plants are the result of the synergistic action of several mechanisms, including antagonism against an array of soilborne pathogens (Benhamou *et al.*, 1999), plant growth promotion through the production of auxin precursors (Le Floch *et al.*, 2003b)

and plant-induced resistance mediated by at least two microbe-associated molecular patterns (MAMPs) (Picard *et al.*, 2000b; Takenaka *et al.*, 2003). A critical feature of the relationships established between *P. oligandrum* and the plant relies on its remarkable ability to rapidly colonize all root tissues in a way similar to pathogenic oomycetes and to subsequently degenerate without causing host tissue damage (Rey *et al.*, 1998b). This unusual behaviour, leading to net fitness benefits for the plant, suggests that *P. oligandrum*, unlike other beneficial micro-organisms (Lorito *et al.*, 2010), does not have the ability to cope with the host immune response that is triggered at the onset of MAMP perception.

In this Review, we provide an overview of the complex and coordinated mechanisms by which *P. oligandrum* protects plants from diseases and interacts with the soil ecosystem. It is also our purpose to discuss the biological principles that drive the action of *P. oligandrum* during its interaction with either plant pathogenic oomycetes or plant root tissues. How *P. oligandrum* differentiates between self and non-self cell wall degradation during mycoparasitism and why it degenerates in the root tissues are two crucial questions that are addressed in this Review. Finally, we discuss future directions of research that would contribute to enhancing the performance of *P. oligandrum* prior to its use as a powerful biocontrol agent.

P. oligandrum: an intruder in the genus *Pythium*

Drechsler (1943) was the first to describe that some *Pythium* species, characterized by their spiny oogonia, were parasites of other pathogenic *Pythium* species. These non-pathogenic plant *Pythium* species were reported as being *Pythium periplocum*, *Pythium acanthicum* and *Pythium oligandrum*. Several years later, Lifshitz *et al.* (1984) found that another *Pythium* species, identified as *Pythium nunn* and isolated from a soil in Colorado, suppressed preemergence damping-off of cucumber seedlings caused by *Pythium ultimum* in greenhouse trials. At the same time, Foley & Deacon (1985) reported that a new *Pythium* species, referred to as *Pythium mycoparasiticum*, was able to parasitize a number of fungal pathogens. Two other spiny oogonial *Pythium* species with mycoparasitic activity, *Pythium acantophoron*, discovered by Lodha & Webster (1990), and *Pythium lycopersicum*, isolated in Turkey (Karaca *et al.*, 2008), have been added to the shortlist of the non-pathogenic *Pythium* species.

Recent advances in our understanding of the biological principles of *P. oligandrum* action have greatly contributed to unravelling the complex machinery developed by this oomycete in relation to its ability to: 1) colonize the rhizosphere of many crop plants and compete for space and nutrients; 2) directly attack an array of soilborne fungal pathogens including ascomycetes (Benhamou *et al.*, 1997; Bradshaw-Smith *et al.*, 1991), basidiomycetes (Ikeda *et al.*, 2012), pathogenic oomycetes (Benhamou *et al.*, 1999) and resting structures (i.e. sclerotia) (Rey *et al.*, 2005); 3) promote plant growth via the putative production of tryptamine (TNH_2), an auxin precursor (Le Floch *et al.*, 2003b); and 4) confer increased crop protection against fungal and bacterial diseases via the activation of the plant immune system (Benhamou *et al.*, 1997; Le Floch *et al.*, 2003a; Takenaka & Tamagake, 2009; Takenaka *et al.*, 2006). In spite of this significant move towards delineating the

lifestyle of *P. oligandrum*, little is known about the communication signals that regulate the interaction of this beneficial oomycete with its ecosystem and with the host plants.

So far, the mechanisms by which effective *P. oligandrum* strains trigger the plant immunity have been studied and at least two types of MAMPs have been discovered in addition to hydrolytic enzymes (Picard *et al.* 2000b; Takenaka *et al.*, 2003). A more comprehensive analysis performed on *P. oligandrum* by the use of the expressed sequence tag (EST) sequencing has recently provided the first overview on the molecules putatively involved in the *P. oligandrum*–prey biochemical cross-talk (Horner *et al.*, 2012). Although of major interest, the data obtained remain speculative and are quite difficult to interpret in the absence of entire genome sequence information. Hopefully, sequencing of the *P. oligandrum* genome in the near future will provide detailed insight into how *P. oligandrum* modulates the plant immune system in such a way that systemic protection in roots and shoots against a broad spectrum of pathogens is conferred.

All beneficial *Pythium* species identified so far are considered to be potential biocontrol agents because of their aggressiveness towards a wide array of soilborne pathogens. However, *P. oligandrum*, probably because it is a common inhabitant in many soils, is the organism that has been the focus of more detailed investigations (Ali, 1985; Ribeiro & Butler, 1992). It is worth mentioning that the antagonism exerted by *P. oligandrum* against pathogenic *Pythium* species represents a rather rare and unique situation in biological control since the biocontrol agent is from the same genus as the pathogen it is controlling (Lévesque, 2011).

Mycoparasitism against fungal and oomycete pathogens

Mycoparasitism can be defined as the ability of a mycoparasite to directly attack a pathogenic fungus and/or an oomycete. Most of our knowledge about the relationships established between non-pathogenic *Pythium* species and their prey derives from a number of cytological and molecular studies that have been conducted to gain a deeper insight into the mechanisms underlying the antagonistic process of *P. oligandrum* (Benhamou *et al.*, 1999; El-Katatny *et al.*, 2006; Horner *et al.*, 2012). A considerable body of work indicates that direct mycoparasitism is a key component of the antagonistic process (Benhamou *et al.*, 1999; Picard *et al.*, 2000a), even though antibiosis and competition for nutrients in the rhizosphere may also account for the observed antagonistic effect against certain preys.

Mycoparasitism is a process that implies an orchestrated scheme of events starting with chemotropic growth of the antagonist towards its microbial prey. In the *P. oligandrum*–fungal and oomycete–pathogen interactions, this early event, likely mediated by extracellular sensing mechanisms, precedes attachment and penetration of the antagonist in the host hyphae (Benhamou *et al.*, 1999; Fig. 1a). Recent investigations into the interaction between *Trichoderma* species and fungal pathogens suggest that peptides, released by the pathogens through the action of proteases produced by the antagonist prior to contact, may bind to specific receptors such as the G protein-coupled receptors or nitrogen-sensing receptors at the plasma membrane level of the antagonist. Once effective binding is achieved, a cascade involving mitogen-activated protein kinases

(MAPKs) (Mukherjee *et al.*, 2003) regulates the activity of transcription factors (currently unidentified) that, in turn, trigger the activation of constitutive genes encoding proteins and enzymes such as cell-wall-degrading enzymes (Druzhinina *et al.*, 2011). Support for this hypothesis comes from the finding that several genes encoding subtilisin-like serine proteases and oligopeptide transporters are overexpressed before and during contact with the prey in at least three *Trichoderma* species that have been sequenced (Seidl *et al.*, 2009). Evidence is also provided that the overexpression of proteases confers an enhanced mycoparasitic activity to these *Trichoderma* strains (Flores *et al.*, 1997). Whether similar mechanisms occur at the onset of contact between *P. oligandrum* and its prey has not yet been elucidated. However, the recent discovery that transcripts encoding cellulases, glucanases, proteases, protease inhibitors, putative effectors and elicitors are produced by *P. oligandrum* grown under biocontrol conditions suggest that *P. oligandrum* operates similarly in the earliest stages of the mycoparasitic process (Horner *et al.*, 2012). Obviously, further work is needed to functionally characterize the potential protease-encoding transcripts identified by Horner *et al.* (2012) before concluding that *P. oligandrum* proteases operate as extracellular sensing mechanisms.

Chemotropism is rapidly followed by the formation of a hyphal network in the immediate vicinity of the fungal prey (Fig. 1). Subsequent events involve cell surface recognition, firm binding of the antagonist to the host cell surface, coiling around the pathogen's hyphae, penetration through the production of hydrolytic enzymes, active multiplication of the antagonistic cells in the pathogen's hyphae and release of the antagonist through moribund hyphal cells (Fig. 2). Recognition events usually involve cell surface molecules from both the antagonist and the pathogen. In the interaction between *P. oligandrum* and *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), a chitin-enriched matrix originating from the pathogen appears to be involved in the recognition process, thus suggesting that peptide receptors with *N*-acetylglucosamine-binding affinity are present at the cell surface of *P. oligandrum* (Benhamou *et al.*, 1999; Fig. 2). Positive correlation between surface-associated components and recognition events in fungal–fungal and oomycete–fungal interactions have often been reported (Benhamou & Chet, 1997) and are considered to be key determinants in the outcome of any mycoparasitic interaction. In their investigation using the green fluorescent protein (GFP) reporter gene, Horner *et al.* (2012) identified a group of very similar transcripts predicted to encode tyrosine- and glycine-rich proteins in *P. oligandrum*. Such proteins are characteristic of cell wall and extracellular matrix proteins and may well collaborate to allow the recognition and attachment of *P. oligandrum* to its prey.

Mycoparasitic attack by *P. oligandrum* usually implies the formation of several papilla-like structures at sites of potential penetration. Enzymic degradation of the host cell wall at these sites facilitates the entry and provides carbon sources required for active growth and development of the antagonist. At the end of the mycoparasitic process, hyphae of the pathogen appear as empty shells with highly altered cell walls and release of *P. oligandrum* cells from these dead host hyphae is frequently observed (Fig. 2). The importance of cell-wall-degrading enzymes is well demonstrated in the cytochemical study by Benhamou *et al.* (1999), which clearly shows that wall-bound chitin

and/or cellulose are severely altered at the onset of *P. oligandrum* penetration. Production of cellulolytic enzymes by *P. oligandrum* has long been the subject of debate because cellulases are hardly detected in culture media containing cellulose or methyl-cellulose as substrates. By contrast, growth of *P. oligandrum* in the presence of isolated oomycete cell walls leads to the massive production of cellulases as shown in the study by Picard *et al.* (2000a). The authors conclusively showed that *Phytophthora* (*Ph.*) *parasitica* hyphae, exposed to the culture filtrate of *P. oligandrum* grown in the presence of *Ph. parasitica* cell walls, exhibited highly altered cell walls. This crucial observation suggests that cellulase synthesis by *P. oligandrum* is a mechanism induced in response to a signal produced by the pathogen itself. The importance of these hydrolytic enzymes in the mycoparasitic activity of *P. oligandrum* is also reflected by the presence of transcripts putatively involved in cell wall degradation (Horner *et al.*, 2012). Indeed, a number of sequences from cDNA libraries of *P. oligandrum* have been annotated as having a key role in the degradation of carbohydrates.

Production of antimicrobial compounds

While mycoparasitism is likely to be the main process by which *P. oligandrum* attacks its prey, production of antimicrobial compounds may, in some interactions, be the only mechanism responsible for pathogen death. The best example is provided by *Phytophthora megasperma* which, under biocontrol conditions, degenerates at a distance from *P. oligandrum* without any cell wall degradation (Benhamou *et al.*, 1999). Clearly, this represents an unusual situation since *Ph. parasitica*, a member of the same genus, is highly vulnerable to the cellulolytic enzymes secreted by *P. oligandrum* at the onset of adhesion to the host hyphae (Picard *et al.*, 2000a). At least two possibilities may explain such a difference. First, *P. oligandrum*, unlike all other known biocontrol agents, may exert a differential mode of action according to its target prey. This, by itself, would indicate that the nature of the communication signals varies from one micro-organism to another, even within the same genus, and that chemotropism, which is a prerequisite for successful mycoparasitism, is species-specific. Second, the target prey may be differentially susceptible to antimicrobial compounds produced by *P. oligandrum* in all circumstances.

Prey defence reactions

Another striking feature of the interaction between *P. oligandrum* and some of its prey concerns the elaboration of structural defence reactions by the pathogen hyphae prior to contact with the antagonist (Benhamou *et al.*, 1999; Picard *et al.*, 2000a). While in most cases *P. oligandrum* is so aggressive that the pathogen does not have time to defend itself from the attack, in other cases, including the *P. oligandrum–Rhizoctonia solani* and the *P. oligandrum–Ph. parasitica* interactions, the pathogen reacts by producing abnormal wall appositions laid down as an attempt to halt entry of the antagonist (Fig. 3). Since such defence reactions are initiated prior to contact between both protagonists and develop further after attachment of the antagonist, it is likely that stress signals are perceived by the pathogens and trigger a cascade of events similar to those known to occur in the plant's defence strategy (Benhamou, 2009). These events may include an oxidative stress response

associated with the production of reactive oxygen species (ROS) resulting in the activation of signalling pathways ultimately leading to the overexpression of genes encoding proteins involved in the synthesis of cell wall compounds. Production of ROS in pathogenic fungi in response to biotic or environmental stress is a mechanism that seems to be required for various physiological processes including hyphal defence under deleterious conditions (Heller & Tudzynski, 2011; Takemoto *et al.*, 2007). Since the genomes of some pathogenic *Pythium* and *Phytophthora* species have been sequenced (Lévesque *et al.*, 2010), the integration of genomic and metabolomic information on *P. oligandrum* will be of considerable value in identifying proteins and metabolites that trigger the perception and response to attack by *P. oligandrum*. Interestingly, host cell wall strengthening does not discourage *P. oligandrum*, which successfully penetrates and invades the reacting host cells, thus confirming its extraordinary ability to massively produce cell-wall-degrading enzymes (Benhamou *et al.*, 1999) (Fig. 3).

Self and non-self cellulose degradation

How *P. oligandrum* differentiates between self and non-self wall-bound cellulose degradation is another key issue that needs to be addressed. Indeed, it remains to be elucidated why cellulases produced by *P. oligandrum* do not harm its own cell walls during the interaction with pathogenic oomycetes. A similar phenomenon occurs during the interaction between some *Trichoderma* species and pathogenic ascomycetes (Lorito *et al.*, 2010). In an attempt to understand the mechanisms underlying such a self–non-self recognition, Gruber & Seidl-Seiboth (2012) proposed a scenario in which the accessibility to the substrate within the fungal cell wall is a key determinant. During *Trichoderma*-mediated mycoparasitism, the fungal pathogen is weakened by a mixture of secondary metabolites and hydrolytic enzymes. This leads to the release of oligosaccharides, which, in turn, stimulate the production of cell-wall-degrading enzymes and accelerate the rate of cell wall degradation, thus giving free access to chitin, the main substrate in fungal pathogens. At the same time, the antagonist may metabolize the released oligosaccharides for remodelling its cell walls. The scenario proposed by Gruber & Seidl-Seiboth (2012) raises the question about the extent to which the cellulolytic enzymes produced by *P. oligandrum* play multiple roles, being used for either the attack of oomycete prey or reconstructing its own cell walls. An answer to this question was partially provided by Horner *et al.* (2012) who discovered that the most frequent clones in the two *P. oligandrum* EST libraries included polysaccharide-degrading enzymes such as cellulases and proteases. Interestingly, one of the cellulases was found to contain a single transmembrane helix, thus suggesting that this enzyme is involved in cell wall synthesis or restructuring. This would explain the extraordinary ability of *P. oligandrum* to readily reconstruct its own cell walls during the mycoparasitic interactions with pathogenic oomycetes.

Intimate interaction with the plant root system: an unusual lifestyle

P. oligandrum strains, considered to be rhizosphere-competent, display the ability to spread into the root tissues without inducing symptoms (Le Floch *et al.*, 2005; Rey *et al.*, 1998a, b). This intimate association is highly beneficial for the plant since it confers increased protection to various biotic

stresses through induced local and systemic resistance and growth promotion via the production of tryptamine. Cytological investigations of *P. oligandrum*-inoculated root tissues show that the antagonist proliferates at the root surface and readily penetrates the epidermis prior to spread within 48 h in all root tissues, including the vascular stele. Probably one of the most intriguing and unusual features of this interaction concerns the sudden degradation of the invading oomycete hyphae during their ingress in the root tissues. This unusual behaviour happens soon after root tissue penetration, as shown by the changes in hyphal structural integrity found to be initiated as soon as 14 h post-inoculation. In the following hours, oomycete cells gradually degenerate to finally become empty walled structures, while typical oogonia arise (Fig. 4). Plant defence reactions, mainly characterized by the formation of discrete wall appositions (Fig. 4), increase over time to reach a peak by 72 h post-inoculation. Additionally, the phenylpropanoid and terpenoid pathways are transiently induced leading to the accumulation of rishitin, a well known phytoalexin, as soon as 14 h post-inoculation (Le Floch *et al.*, 2005). Since accumulation of newly formed phenolics proceeds at a time when structural changes in *P. oligandrum* hyphae start to be visible, it seems reasonable to assume that a positive correlation exists between plant defence reactions and *P. oligandrum* hyphal alteration.

To some extent, the mode of action of *P. oligandrum* resembles that of *Trichoderma* strains which have been described as being opportunistic symbiotic fungi (Trillas & Segarra, 2009), capable of not only colonizing the outermost root tissues without causing extensive damage but also stimulating plant growth and triggering plant defence reactions (Yedidia *et al.*, 2000, 2001). Several lines of evidence indicate that beneficial micro-organisms are initially recognized by the plant as potential invaders, after which a defence response is triggered. Later on, mutualists cope with the host immune response, allowing them to stay alive in the plant tissues. The fact that the invading hyphae of *P. oligandrum* degenerate concomitantly with the accumulation of plant defence reactions strongly suggests that *P. oligandrum* is not able to short-circuit plant defence responses as do most other mutualistic agents through the production of effector-like molecules (Plett *et al.*, 2011; Zamioudis & Pieterse, 2012). Undoubtedly, genome sequencing of *P. oligandrum* will expand our knowledge of the mechanisms involved in the unusual relationships that this oomycete establishes with the plant.

Plant growth promotion mediated by tryptamine

Growth enhancement by beneficial micro-organisms is a well-documented phenomenon that has often been associated with the synthesis of microbial phytohormones and secondary metabolites (Helman *et al.*, 2011; Hermosa *et al.*, 2012).

Le Floch *et al.* (2003b) elucidated some of the key mechanisms underlying plant growth promotion by *P. oligandrum*. The finding that large amounts of tryptamine (TNH_2) were produced when *P. oligandrum* was grown in a culture medium amended with auxin precursors, including tryptophan (Trp) and indole-3-acetaldehyde (IAAld), was taken as an indication that *P. oligandrum* produced tryptamine (TNH_2), an auxin-like compound. Such a pathway is well known to operate in a number

of non-pathogenic fungi (Frankenberger & Arshad, 1995) as well as in *P. ultimum* and *Pythium* group F (Rey *et al.*, 2001). The difference, however, relies on the ability of these fungi and oomycetes to convert TNH_2 into indole-3-acetic acid (IAA), a process that does not seem to be operational in *P. oligandrum*. Interestingly, Le Floch *et al.* (2003b) found that TNH_2, formed following conversion of Trp in the plant nutrient solutions amended with *P. oligandrum*, was readily adsorbed by the root system, resulting in an increase in root weight associated with an enhanced formation of secondary roots.

A tryptamine pathway, similar to that found in certain fungi, exists in tomato plants (Cooney & Nonhebel, 1991). Although TNH_2 is not a major endogenous precursor of IAA in tomato shoots, it is likely that a moderate TNH_2 influx from an external origin can trigger the synthesis of IAA, leading to increased tomato plant growth. Thus, TNH_2, secreted by *P. oligandrum* in the rhizosphere, is likely absorbed by the root system and converted into IAA that, in turn, amplifies plant growth.

One critical question in the study of *P. oligandrum*-mediated plant growth promotion was to determine how the oomycete could produce TNH_2 in the rhizosphere. Evidence was provided that, in a way similar to other soilborne micro-organisms, *P. oligandrum* used root exudates as nutrient sources. Provided that such nutrient sources contain precursors such as Trp, the antagonist is then able to produce TNH_2. Since Trp has been detected in root exudates from some tomato cultivars (Rybicka, 1981) and since roots are sensitive to very low concentrations of auxins (Taiz & Zeiger, 1998), it is likely that a slight but frequent production of TNH_2 by *P. oligandrum* in the rhizosphere exerts a beneficial effect on the plant physiology.

Plant-induced resistance mediated by MAMPs

Plants possess an ‘immune system’ that can be stimulated by specific signals originating from a potential aggressor (Benhamou, 2009). Immune signalling in plants has been investigated in depth for a number of plant–pathogen interactions and it is generally acknowledged that two key events account for the establishment of successful resistance: 1) receptor-mediated perception of pathogen-associated molecular patterns (PAMPs); and 2) response to virulence factors from the pathogen, the so-called effector-triggered immunity (ETI) (Jones & Dangl, 2006). Non-pathogenic micro-organisms that display the ability to penetrate the plant may also trigger the plant immune system through the recognition of MAMPs that sensitize the plant to respond more efficiently to subsequent pathogen attack (Druzhinina *et al.*, 2011).

P. oligandrum-mediated induced resistance

P. oligandrum protects plants from subsequent infection by a pathogen, as do other biocontrol agents (Veloso & Diaz, 2012). *P. oligandrum*-mediated induced resistance is associated with marked host metabolic changes culminating in a number of physical and biochemical responses involved in restricting pathogen penetration and development in the host tissues (Benhamou *et al.*, 1997) either indirectly (reinforcement of plant cell walls) or directly (antimicrobial activity). As shown in the tomato–FORL interaction, the *de novo* formation of callose-enriched wall appositions

is apparently efficient at preventing pathogen ingress towards the vascular stele and probably also in shielding the inner root tissues from phytotoxic, diffusible products such as hydrolytic enzymes and toxins (Fig. 5). The release of phytoalexins and the *de novo* synthesis of pathogenesis-related (PR) proteins (i.e. chitinases and β-glucanases) may also account for the observed degradation of FORL hyphae in the root tissues. Another example that confirms the potential of *P. oligandrum* for inducing local resistance against soilborne pathogens is provided by the enhanced tomato protection against *Ralstonia solanacearum*, the root pathogenic bacterium responsible for lethal wilting disease in over 200 different plant species (Genin & Denny, 2012). In *P. oligandrum*-inoculated tomato plants, bacterial spread in the root tissues is remarkably halted by structural plant defence mechanisms (Masunaka *et al.*, 2009). In addition to induction of local resistance against fungal, oomycete and bacterial pathogens, *P. oligandrum* is also able to trigger systemic induced resistance, as observed in grapevine and tomato infected by *Botrytis cinerea*, the agent of grey mould (Le Floch *et al.*, 2003a; Mohamed *et al.*, 2007). The increase in β-1,3-glucanase and stilbene synthase transcripts, as shown by RT-PCR, confirms the potential for *P. oligandrum* to trigger the synthesis and accumulation of defence-related molecules (i.e. PR proteins and phenolics), likely responsible for the creation of an environment adversely affecting pathogen viability.

P. oligandrum-derived MAMPs

Two types of MAMPs have been clearly identified so far. The first recognized *P. oligandrum* MAMP was oligandrin, a 10 kDa protein exhibiting to some extent the typical elicitin 'signature' described by Ponchet *et al.* (1999). Examination of the N-terminal sequence alignment with sequences from 13 elicitins secreted by some *Phytophthora* and *Pythium* species revealed that oligandrin was actually an elicitin-like protein harbouring original features than a true elicitin (Picard *et al.*, 2000b). Support for this concept was provided by the observation that oligandrin infiltration into tomato leaves failed to mediate the hypersensitive reaction (HR)-associated necrotic response, a reaction consistently found to occur in tobacco plants treated with true elicitins (Ponchet *et al.*, 1999). In spite of such differences, the high level of oligandrin-mediated protection obtained against *Ph. parasitica* (Picard *et al.*, 2000b) and FORL (Benhamou *et al.*, 2001) in tomato, *Phytoplasma* in tobacco (Lherminier *et al.*, 2003) and *B. cinerea* in grapevine (Mohamed *et al.*, 2007) supports the conclusion that oligandrin is a powerful, but not a specific elicitor of resistance.

The qualitative response of tomato plants to oligandrin differs according to the challenging pathogen. In the tomato–*Ph. parasitica* interaction, oligandrin strongly stimulates the phenylpropanoid and terpenoid pathways, leading to an increased accumulation of phenolic compounds, which, in turn, affect pathogen cell viability (Fig. 6). By contrast, structural defence reactions are not or are only slightly induced in these oligandrin-treated tomato plants (Picard *et al.*, 2000b). In the tomato–FORL interaction, oligandrin induces a massive deposition of wall appositions at sites of potential pathogen penetration in addition to also triggering the synthesis and accumulation of antifungal compounds (Fig. 6). In tobacco, oligandrin triggers the elaboration of an array of plant defence responses, including impregnation of cell walls with phenolic compounds, formation of calcium pectate gels in intercellular spaces and accumulation of newly synthesized

proteins in phloem bundles (Lherminier *et al.*, 2003). Similar plant defence responses are seen in oligandrin-treated grapevine challenged with *B. cinerea* (Mohamed *et al.*, 2007). Together, the observations made on tomato, tobacco and grapevine plants highlight that oligandrin triggers the activation of defence genes, the expression of which may be modulated according to the target plant pathogen.

P. oligandrum cell wall glycoproteins (CWPs), also called POD-1 and POD-2, can also act as potent elicitors (Takenaka *et al.*, 2006). The amino sequences deduced from the corresponding cDNA sequences of POD-1 and POD-2 (DDBJ accession nos AB217820 and AB217821, respectively) exhibit an elicitin 'signature' as well as *O*-linked glycosylation sites anchoring the proteins to the cell wall (Takenaka *et al.*, 2006). Indeed, these sequences reveal the occurrence of a conserved region with six cysteine residues similar to that found in true elicitins and oligandrin. Together with oligandrin, POD-1 and POD-2 are classified among the elicitin-like proteins based on their ability to stimulate the plant defence strategy without inducing a typical HR. However, evidence is provided from a recent study that POD-1 and POD-2 form a specific heterohexamer whose intact 3D structure is needed for elicitor activity (Takenaka *et al.*, 2011).

Recent molecular investigations on the distribution and expression of oligandrin and CWP genes among 10 *P. oligandrum* isolates showed that two CWP genes (*pod-1* and *pod-2*) and two oligandrin genes (*oli-d1* and *oli-d2*) occurred as single copies and were present in the *P. oligandrum* genome of all tested isolates but not in the genomes of other *Pythium* species (Masunaka *et al.*, 2010). These results indicate that the elicitin-like proteins of *P. oligandrum* are species-specific. Interestingly, RT-PCR analyses with gene-specific primers indicate that these specific genes encoding elicitin-like proteins are highly expressed upon colonization of tomato root tissues by *P. oligandrum*, thus supporting the concept that the two types of elicitin-like proteins are secreted *in planta*. Similar to oligandrin, CWP fractions, applied either as root drench or foliar spray, confer increased plant protection against fungal and bacterial pathogens (Takenaka & Tamagake, 2009). This induced protection correlates with the activation of plant defence genes, leading to the synthesis and accumulation of defence molecules including phenylalanine ammonia lyase (PAL), a key enzyme involved in the phenylpropanoid pathway, basic PR proteins, and cell-wall-bound phenolic compounds (Takenaka *et al.*, 2003).

In addition to oligandrin and CWPs, the possibility that cellulases, abundantly produced by *P. oligandrum*, may act as MAMPs is presently under consideration. That cellulases may operate as inducers of defence reactions is a concept that has already been shown in *Trichoderma*-mediated plant-induced resistance (Martinez *et al.*, 2001). Other than through MAMPs, micro-organisms may also be detected via damage-associated molecular patterns, which are endogenous plant-derived molecules (i.e. pectin compounds) that arise from enzymic degradation of cell walls. Interestingly, Horner *et al.* (2012) found that transcripts putatively encoding pectinases occurred in the *P. oligandrum* libraries, thus suggesting that pectin residues, released from the plant cell walls during ingress into the root tissues, may also operate as potential elicitors. Thus, *P. oligandrum* possesses a pallet of mechanisms able to trigger the plant immunity. However, it seems likely that, unlike other

beneficial microbes, *P. oligandrum* cannot repress the defence genes that are stimulated upon MAMP perception.

Signalling pathways involved in *P. oligandrum*-mediated induced resistance

While the signal transduction pathway conferring oligandrin-mediated induced resistance has not yet been elucidated, data regarding the signalling network involved in the CWP-mediated induced resistance indicate that the jasmonic acid (JA)- and ethylene (ET)-dependent signalling pathways play a synergistic role in the plant defence response (Hase *et al.*, 2006, 2008). SA, the hormone known to be associated with HR and systemic acquired resistance (SAR) against a wide range of biotrophic pathogens (Vlot *et al.*, 2009), does not seem to be involved in the resistance induction process as shown by the finding that treatment of tomato plants with CWPs failed to induce an accumulation of SA or a production of the SA-responsive PR-1 proteins (Hase *et al.*, 2008; Takahashi *et al.*, 2006). Furthermore, global gene expression analysis using a tomato cDNA array indicates that SA-responsive genes are not upregulated following CWP treatment (Takahashi *et al.*, 2006).

Cross-talk between SA- and JA-signalling networks plays a key role in the regulation of induced plant defence against pathogens by exerting antagonistic effects (Sendon *et al.*, 2011). Because induced resistance to *Ralstonia solanacearum* is not compromised in CWP-treated *nahG* transgenic tomato mutants (transgenic plants expressing the bacterial salicylate hydroxylase gene) while it is compromised in *jai1-1* mutants with an impaired JA signalling pathway (Hase *et al.*, 2008), it is tempting to speculate that, analogous to other beneficial associations (Van der Ent *et al.*, 2009), the JA-dependent signalling pathway is required in the CWP-mediated induced resistance. The recent use of an array of mutants with different impaired defence signalling pathways brought conclusive evidence for an involvement of the JA- and ET-dependent signalling pathways in the CWP-induced plant response (Kawamura *et al.*, 2009). It seems likely that the strong activation of the JA signalling pathway by CWPs from *P. oligandrum* results in the suppression of the SA signalling pathway through hormonal cross-talk mechanisms.

Rhizosphere competence and interactions with the indigenous microflora

The rhizosphere microbial community plays a major role in ecosystem functions and is among the most complex and diverse community in the biosphere. Microbial diversity in the rhizosphere is linked to plant species mainly because interactions between root exudates and soil micro-organisms are highly dynamic in nature and based on co-evolutionary pressures (Broeckling *et al.*, 2008). In the past few years, only a few studies have focused on the impact of biocontrol agents (Cordier & Alabouvette, 2009; Vallance *et al.*, 2011) on the structure and function of the rhizosphere microbiome. Although *P. oligandrum* is highly effective in inducing local and systemic plant disease resistance, the question about the extent to which its introduction in soils may influence the

growth of non-target species, including saprophytic and beneficial fungi and rhizobacteria, is of prime importance prior to potential commercialization.

Rhizosphere competence and effect on the rhizosphere microbial community

The ability of *P. oligandrum* to be a strong colonizer of the rhizosphere has been the subject of many debates (Al-Rawahi & Hancock, 1997; McQuilken *et al.*, 1990) until the advent of modern molecular approaches such as real-time PCR and DNA macroarray. All data collected so far indicate that *P. oligandrum* competes in the rhizosphere for space (i.e. root zone niches or attachment) and nutrients with native soil microflora and develops over the tomato root surface (Le Floch *et al.*, 2007; Takenaka *et al.*, 2008).

The possibility of amending nutrient solutions with selected strains of *P. oligandrum* has been assessed by Vallance *et al.* (2009). Results from single-strand conformational polymorphism (SSCP) fingerprinting analyses provide evidence that an increase in the complexity and size of the microflora, likely due to the attraction of fungal populations by root exudates, occurs with time. This observation supports the view that *P. oligandrum* is able to persist in the rhizosphere even when the latter is colonized by a complex and changing microflora.

In spite of its ability to grow and persist in the rhizosphere, *P. oligandrum* does not induce significant shifts in the rhizosphere fungal microflora. Indeed, it does not modify the indigenous fungal populations, other than a reduction of the population of pathogenic *P. dissotocum* in the tomato rhizosphere (Vallance *et al.*, 2009). The influence exerted by *P. oligandrum* on the bacterial populations proliferating in the rhizosphere of tomato plants grown in a hydroponic system is also not quantitatively affected by the presence of *P. oligandrum* (Vallance *et al.*, 2012). Indeed, the introduction of *P. oligandrum* in soil-less growing systems does not necessarily result in significant perturbations of the bacterial microflora, as shown by the absence of evolution in the overall number of bacteria developing around the root system of tomato plants during the growing season. Only transient perturbations in the indigenous bacterial communities are detected at the onset of *P. oligandrum* inoculation in the rhizosphere. This shift in bacterial communities is, however, transient since it gradually decreases to become negligible at the end of the cropping season. Thus, *P. oligandrum*, in a way similar to other biocontrol agents including the non-pathogenic *Fusarium oxysporum* strains Fo47 (Edel-Hermann *et al.*, 2009) and FsK (Karpouzas *et al.*, 2011), induces slight or transient changes in the fungal and bacterial communities, highlighting the absence of undesirable effects on the diversity of non-target rhizosphere microbial groups.

Interaction with other biocontrol agents

As for other beneficial micro-organisms, field survival and persistence is probably the main limitation for the effective use of *P. oligandrum*. In this respect, adverse environmental conditions – such as non-optimal temperature and humidity parameters and competitive displacement of the antagonist by endogenous rhizosphere micro-organisms that occupy the same niche and reduce nutrient availability – may greatly affect survival of *P. oligandrum* or, at least, reduce its beneficial

effects. One option that is attracting much attention concerns the possibility of creating new combinations of beneficial micro-organisms with complementary modes of action. In the last few years, several reports have shown that co-inoculation of beneficial micro-organisms could stimulate plant growth and/or increase plant disease resistance relative to inoculation with a single biocontrol agent (Raimam *et al.*, 2007; Whipps, 2004). Although such combinations may enhance the level of plant protection against pathogen attack (Ezziyyani *et al.*, 2007; Guetsky *et al.*, 2001, 2002), the possible competitiveness between these micro-organisms has to be taken into consideration (Alabouvette *et al.*, 2006).

An approach combining *P. oligandrum* with two other well-documented biocontrol agents (*T. harzianum* and *Fusarium oxysporum*, strain Fo47; Benhamou *et al.*, 2002; Harman, 2006), reveals that *P. oligandrum* successfully colonizes the rhizosphere and is able to penetrate the root system (Le Floch *et al.*, 2009). Additionally, the observations indicate that *P. oligandrum*, alone or in combination with the two other antagonists, is similarly effective at reducing grey mould incidence in tomato plants infected by *B. cinerea* and that this increased protection apparently correlates with an overexpression of protein PR-3 (chitinases). It is likely that *P. oligandrum*, in a way similar to plant-growth-promoting bacteria (Benhamou *et al.*, 1996) and *Trichoderma* species (Harman *et al.*, 2004; Yedidia *et al.*, 2003), exerts a priming effect that is fully expressed when the plant is subsequently challenged by a pathogen.

The work of Le Floch *et al.* (2009) provides support to the concept that co-inoculation of beneficial organisms does not necessarily lead to amplified synergistic effects. It further highlights that, from an ecophysiological viewpoint, our knowledge of the interactions between beneficial micro-organisms is rudimentary.

Conclusions and future perspectives

Although oomycetes are generally viewed as aggressive plant pathogens causing severe yield losses to cultivated crops, this Review demonstrates that some members of this group of diploid micro-organisms have a remarkable potential as bio-inoculants with respect to their ability to promote plant growth, induce plant protection and exert antimicrobial effects on a wide range of plant pathogens. The expanding knowledge of the mechanisms underlying the mode of action of *P. oligandrum* will open new avenues of potential applications. Currently, only one *P. oligandrum*-based agricultural product, Polyversum (Brozova, 2002), is registered but it is likely that other *P. oligandrum*-derived products will be commercialized in the near future.

Recent advances in proteomics, genomics and metabolomics have been instrumental for identifying and characterizing the signalling molecules involved in the molecular dialogue between *P. oligandrum* and its hosts. Some of these molecules act as auxin-like compounds such as tryptamine (TNH_2), an auxin-compound which, upon root absorption, stimulates plant growth (Le Floch *et al.*, 2003b). Other molecules like oligandrin (Picard *et al.*, 2000b) and CWPs (Takenaka *et al.*, 2003) operate as elicitors of plant disease resistance and trigger defence gene expression through the activation of JA- and ET-mediated signalling pathways (Hase *et al.*, 2006, 2008). Finally, the

antifungal arsenal of *P. oligandrum* includes a battery of lytic enzymes (chitinases, glucanases, cellulases) that likely play a key role by allowing the release of cell-wall-bound oligosaccharides. Recent studies on the proteome of *Trichoderma* spp. have provided novel data regarding the oligosaccharides released through the action of lytic enzymes during mycoparasitim (Woo *et al.*, 2006; Woo & Lorito, 2007). Such oligosaccharides, originating from the prey cell walls, appear to be essential for stimulating *Trichoderma* growth and triggering the production of antibiotics and lytic enzymes in addition to acting as elicitors of the plant defence response (Vinale *et al.*, 2008). Apart from oligosaccharides, other molecules such as peptaibols are thought to contribute to the activation of the plant defence strategy by *Trichoderma* species (Benítez *et al.*, 2004; Djonović *et al.*, 2006). The data on the ability of *Trichoderma* species to produce an array of potential elicitors raise the question about the extent to which the enzymes secreted by *P. oligandrum* are involved in the release of molecules with potent elicitor activity. Similarly, very little is known about the ability of this beneficial oomycete to produce secondary metabolites. Obviously, further research is needed to deeply explore the *P. oligandrum* proteome, genome and metabolome. This would also greatly contribute to determining whether *avr*-like genes exist in *P. oligandrum* and are involved in induced resistance and recognition of the antagonist by the plant, as has been suggested for *Trichoderma* species (Woo & Lorito, 2007). Recently, a variety of ABC transporters has been associated with the ability of *Trichoderma* species to withstand the impact of toxins produced by pathogens and plants (Woo & Lorito, 2007). Interestingly, recent data obtained from the first *P. oligandrum* EST libraries indicate the presence of ABC transporter-related homologues which may be involved in either the transport of toxic compounds out of the cytoplasm or the secretion of virulence factors. Other sequences with possible defence-related functions include xenobiotic reductases and proteins involved in the detoxification of ROS, such as glutathione transferases and thioredoxin peroxidase (Horner *et al.*, 2012). This preliminary information is of prime importance since it highlights, for the first time to our knowledge, that *P. oligandrum* has the necessary machinery to protect itself against harmful pathogen or plant metabolites.

What we have learned from all the studies conducted so far on the ability of *P. oligandrum* to colonize the rhizosphere is that it is generally a poor competitor, although rhizosphere colonization appears to depend upon the culture system used. Whereas competition for nutrients, space and infection sites is one of the major mechanisms that drives the biocontrol activity of both *Trichoderma* species and non-pathogenic *Fusarium* strains (Alabouvette *et al.*, 2009; Benítez *et al.*, 2004), a growing body of evidence from French and Japanese studies tends to indicate that the biocontrol efficacy of most strains of *P. oligandrum* does not rely on competition for nutrients and space but rather depends on their ability to induce resistance (Takenaka *et al.*, 2008). However, it is clear that to perform, any biocontrol agent should be able to grow and develop in the rhizosphere in order to reach the population level needed to be effective. In that context, functional genomic studies would be very useful for improving the selection of performing strains. These approaches have already proven helpful for: 1) identifying the relevant genes and their expression pattern in *Trichoderma* strains under various conditions; 2) delineating the molecular mechanisms that mediate the higher activity both outside (mycoparasitism, antibiosis, etc.) and inside (plant growth

promotion, plant disease resistance) the plant; and 3) characterizing the *Trichoderma* strains recommended for use either alone or in combination (Lorito *et al.*, 2010). The use of these molecular techniques would certainly help in selecting the best *P. oligandrum* strains and proposing formulations with optimal effects in terms of plant protection and plant growth promotion. The other approach investigated for improving the efficacy of *P. oligandrum* concerns its combination with other biocontrol agents selected on the basis of their well-documented performance (Le Floch *et al.*, 2009). In the presence of *T. harzianum* and *F. oxysporum* Fo47, *P. oligandrum* is a poor competitor although it can survive at a distance from its partners. The observation that the plant response to *P. oligandrum* alone is not substantially enhanced when the oomycete is associated with one or both of the other fungal antagonists is intriguing, although one may be tempted to suggest that the priming effect exerted by *P. oligandrum* is optimal and, therefore, cannot be amplified regardless of the presence of other beneficial micro-organisms. Finally, an issue that has not yet been addressed would consist of manipulating selected strains of *P. oligandrum* in such a way that their biocontrol activity is amplified. Such a strategy has already proved successful for overexpressing genes encoding endo- and exochitinases in *Trichoderma* strains (Zeilinger *et al.*, 1999) as well as genes encoding precursors of phenazin, an antifungal compound produced by *P. fluorescens* (Huang *et al.*, 2004). Functional genomic studies and other approaches such as *in vivo* expression technology (Silby & Levy, 2004) would also be helpful for providing an insight into genes that are required by *P. oligandrum* to compete in the rhizosphere, to exert mycoparasitism and to colonize the plant root tissues.

In *P. oligandrum*-primed plants, local and systemic defence responses are accelerated upon pathogen attack, resulting in enhanced resistance and increased crop yield. Two types of elicitors or MAMPs, oligandrin and CWPs, have been identified so far that play a crucial role in the onset of the plant defence response (Picard *et al.*, 2000b; Takenaka *et al.*, 2006). In *Arabidopsis*, evidence has been provided that resistance mediated by beneficial rhizobia is linked with priming for enhanced expression of ET/JA-responsive genes, followed by formation of wall appositions and accumulation of phenolic compounds upon pathogen attack (Ahn *et al.*, 2007). Similarly, plants inoculated with *Trichoderma* strains (e.g. *Trichoderma asperellum*) develop an ET/JA-dependent systemic resistance that is subsequently characterized by an overexpression of PR genes following pathogen challenge (Shoresh *et al.*, 2005). It thus appears that ET and JA serve as the endogenous signals in the beneficial micro-organism-induced primed state. The early signalling events induced by *P. oligandrum* or its elicitor, oligandrin, have not yet been explored, although there is good reason to believe that they follow a typical scheme including ROS production, ion fluxes, protein phosphorylation and protein kinase activation (Garcia-Brugger *et al.*, 2006).

All research data on *P. oligandrum* reveal that its behaviour is similar, in most respects, to that of *Trichoderma* species since it provides many benefits to the host and uses the root exudates as food sources. However, the observation that *P. oligandrum* hyphae degenerate soon after their entry in the root tissues excludes the possibility that it may be considered as a symbiotic micro-organism. By contrast, its ability to promote plant growth and improve plant performance suggests that it is a

plant-growth-promoting oomycete which can stimulate the plant immune system before degenerating and producing oogonia. Until we understand more about the biological significance of *P. oligandrum* hyphal degradation in the plant root tissues, our knowledge of this unique relationship will be limited.

Because plant diseases cause billions of dollars of harvest loss annually with huge consequences in developing countries, the development of management strategies that can be reliable and safe for the environment is urgently needed. Today, the emergence of resistant pathogen strains to currently used pesticides, the increasing consumer demand for food products without toxic residues and the international objective to preserve as much as possible the environment and human health, reduce the use of pesticides and favour the introduction of novel alternatives. Among these, activating plant defence responses by using biocontrol agents or their released elicitors is a promising strategy for replacing, or at least reducing, chemical applications. As we learn more about the contribution of *P. oligandrum* in plant growth and protection, it will be possible to amplify its performance and facilitate its implementation in crop management. Because the beneficial effects exerted by *P. oligandrum* itself can be replicated by using MAMPs, it is reasonable to speculate that new formulations based on bioactive metabolites will be commercialized in the near future.

Acknowledgements

We acknowledge funding received from the Fonds de Recherche du Québec-Nature et Technologies (FQRNT), the Natural Sciences and Engineering Research Council of Canada (NSERC) as well as the Brittany and Pays de la Loire Regional Councils.

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Fig. 1. Interaction between *P. oligandrum* (Po) and *Ph. parasitica* (Ph) in confrontation tests at 24 h post-inoculation. (a) Light microscopy. Chemotropism, as illustrated by the attraction of numerous *P. oligandrum* hyphae towards a cell of *Ph. parasitica*. (b) Transmission electron microscopy. Attachment and adhesion of *P. oligandrum* hyphae at the cell surface of a *Ph. parasitica* cell. Bars, xxx (a), xxx (b). Part (b) was published in Picard *et al.* (2000a) and is reproduced with permission from the American Society for Microbiology (ASM).

Fig. 2. Illustration of the main events occurring in the interaction between *P. oligandrum* (Po) and FORL. (1) Early contact between *P. oligandrum* and *Fusarium* hyphae is mediated by an amorphous chitin-enriched matrix (Ma). (2) Coiling of *P. oligandrum* around a *Fusarium* hypha. The coils tightly encircle the host hypha (large arrow). Penetration through an appressorium-like structure is visible (small arrow). (3) Penetration of the antagonist in the prey hypha via production of cell-wall-degrading enzymes. (4) Active multiplication of *P. oligandrum* hyphae inside the *Fusarium* hypha. (5) Release of *P. oligandrum* hyphae through the moribund FORL hypha. Bars, xxx. Pictures 1, 2 and 4 were published in Benhamou *et al.* (1999) and are reproduced with permission from the American Society for Microbiology (ASM).

Fig. 3. Features of defence reactions elicited by *Ph. parasitica* (Ph) in response to *P. oligandrum* (Po) attack. A. The cell wall of a *Ph. parasitica* hypha is markedly thickened at a time when contact with the antagonist happens. Upon adhesion of *P. oligandrum*, cellulolytic enzymes are produced and breaching of the cell wall outer layers is visible (arrow). (b) Hyphae of *P. oligandrum* start to degrade a highly thickened host cell wall through the production of large amounts of cellulolytic enzymes (arrow). (c) At a later stage, *P. oligandrum* successfully penetrates the thickened cell wall of a responsive *Ph. parasitica* hypha (arrows) and rapidly spreads into the prey cell. Labelling of the host cell wall for cellulose localization was performed with an exoglucanase–gold complex. Bars, xxx. Parts (b and c) were published in Picard *et al.* (2000a) and are reproduced with permission from the American Society for Microbiology (ASM).

Fig. 4. Scheme of the events occurring during the interaction between *P. oligandrum* (Po) and tomato root tissues. (1) and (2): Following inoculation of *P. oligandrum*, the first events include development of the antagonist at the root cell surface prior to penetration in the root epidermis and rapid dissemination into the inner tissues towards the vascular stele. By 9 h post-inoculation, hyphae abundantly colonize the cortex through local host cell wall penetration. At that time, hyphae of the antagonist appear metabolically active as judged by their dense cytoplasm. (3) By 14 h post-inoculation, hyphae of the antagonist undergo structural changes, characterized by increased vacuolation and cytoplasm disintegration. The phenylpropanoid and terpenoid pathways begin to be activated. (4) By 48 h post-inoculation, antagonistic cells appear as empty shells surrounded by enlarged oogonia (Oo). (5) By 72 h post-inoculation, the alteration of *P. oligandrum* hyphae in the root tissues coincides with the development of host defence responses, including the formation of wall appositions (WA). Bars, xxxx. Images illustrating the events 2 and 3 were published in Le Floch *et al.* (2005) and are reproduced with permission from Elsevier. The image illustrating the event in 5 was published in Benhamou *et al.* (1997) and is reproduced with permission from the American Phytopathological Society (APS).

Fig. 5. Light microscope (a, b) and electron microscope (c, d) images of tomato root tissues infected with FORL (F). (a) Control plants. Hyphae of the pathogen (F) multiply in the epidermis (Ep) and the cortex (Co), and reach the vascular stele (VS). The cortical tissue is highly degraded as illustrated by the near absence of visible cell wall structures. (b–d) Plants inoculated with *P. oligandrum* and challenged with FORL. Fungal pathogen growth is mainly restricted to the epidermis (Ep) and the outer root cortex (Co). Restriction of pathogen spread is associated with the deposition of electron-dense granules, likely enriched in phenolics, in the intercellular spaces (c, white arrow) and with the formation of wall appositions (WA) at sites of potential host cell wall (HCW) penetration by the pathogen (d). Bars, xxx.

Fig. 6. Effect of oligandrin, a *P. oligandrum*-produced MAMP, on the colonization of tomato plants by *Ph. parasitica* (Ph) (a–c) and FORL (F) (d–f). (a) Control plants infected with *Ph. parasitica*. *Phytophthora* hyphae appear metabolically active and the pathogen rapidly colonizes the leaf and stem tissues through penetration of the host cell walls (HCW). (b, c) Plants treated with oligandrin and infected with *Ph. parasitica.* Most of the pathogen hyphae (Ph) are highly disorganized as shown by their aggregated cytoplasm which is filled with electron-dense inclusions (b). Some other invading hyphae are highly altered (c). The host cell wall is apparently well preserved. Bars, xxx. (d) Control plants infected with FORL. The pathogen spreads rapidly in all root tissues and produces large amounts of hydrolytic enzymes that degrade the host cell wall (arrow). (e, f) Plants treated with oligandrin and infected with FORL. Defense reactions, designed to halt pathogen ingress, are characterized by the accumulation of aggregated deposits that coat hyphae of the pathogen in the intercellular spaces (e, f; arrow). Wall appositions are formed along the host cell walls (e, f). Bars, xxx. (b) was published in Picard *et al.* (2000b) and is reproduced with permission from the American Society of Plant Biologists. (e) was published in Benhamou *et al.* (2001) and is reproduced with permission from Elsevier.

Figure 1

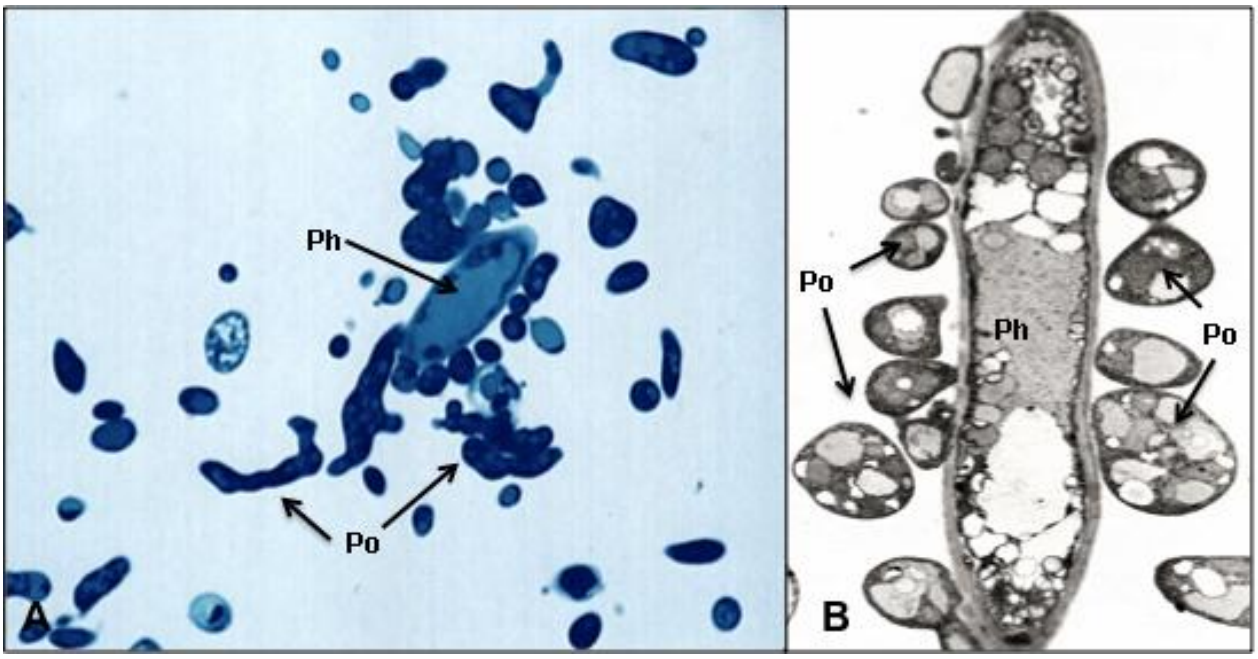

Figure 2

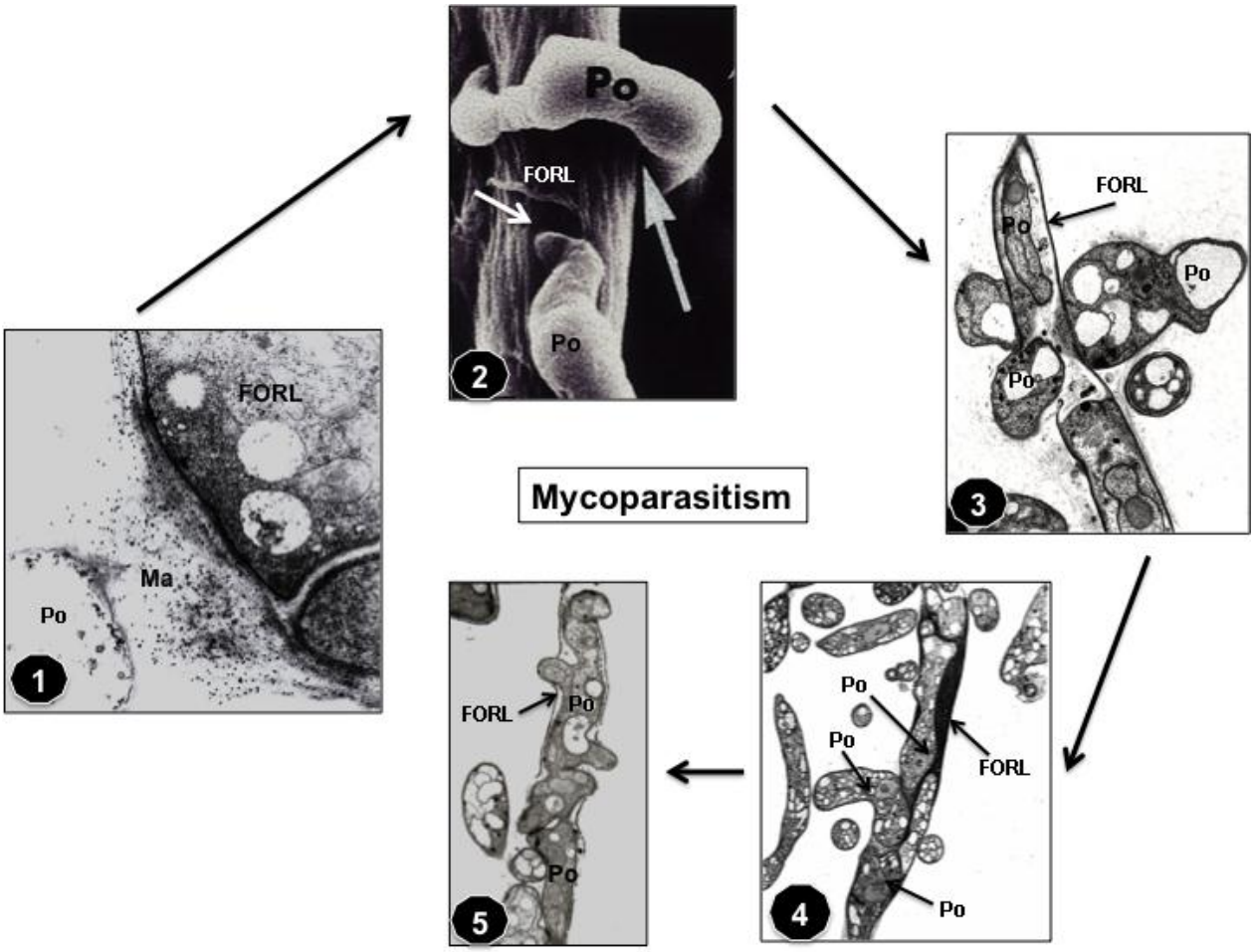
Po
FORL
Po
2
FORL
Po
Po
Po
3
Mycoparasitism
FORL
Ma
Po
1
Po
Po
FORL
4
FORL
Po
Po
5

Figure 3

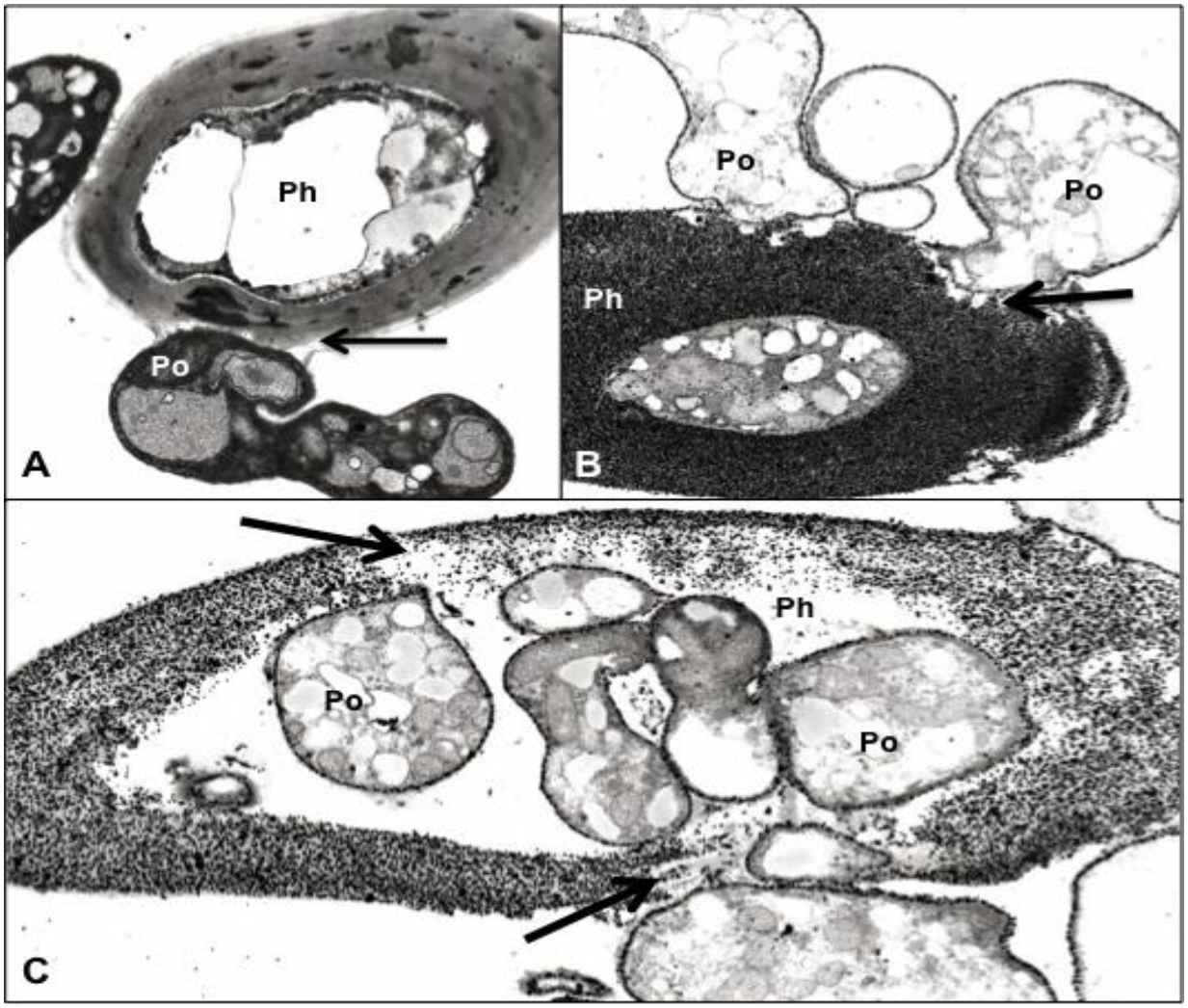

Figure 4

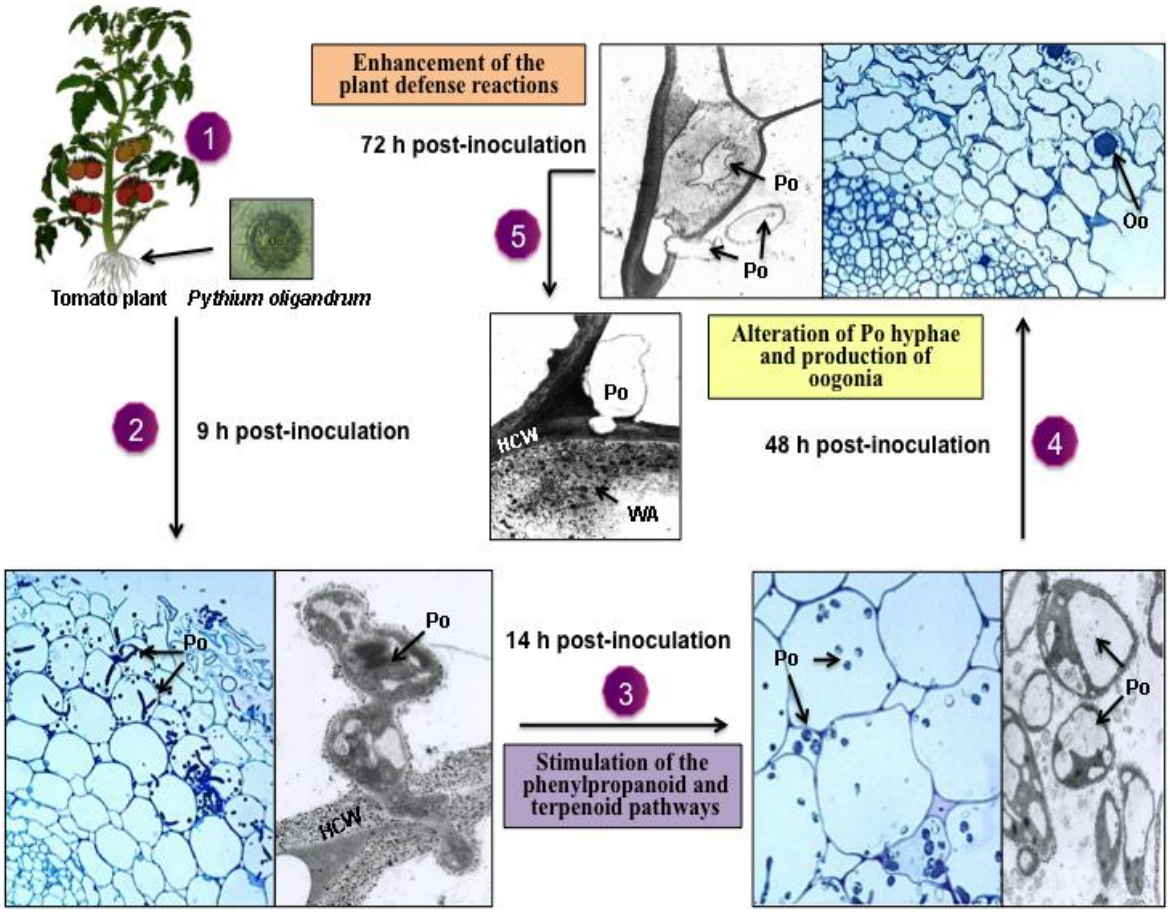
Tomato plant
Pythium oligandrum
1
2
9 h post-inoculation
3
14 h post-inoculation
Stimulation of the phenylpropanoid and terpenoid pathways
4
48 h post-inoculation
Alteration of Po hyphae and production of oogonia
5
72 h post-inoculation
Enhancement of the plant defense reactions
Po
Oo
HCW
WA

Figure 5

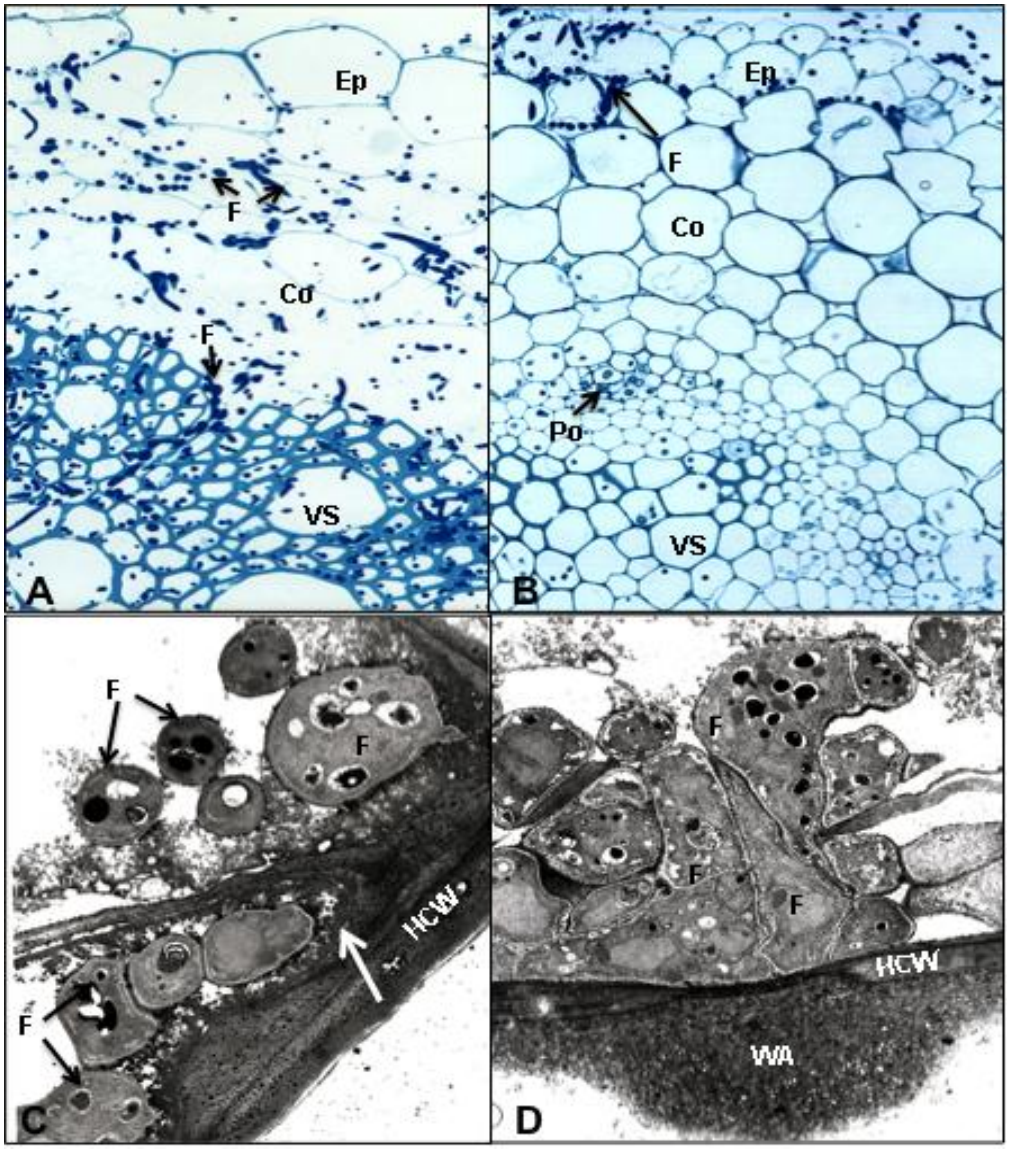

Figure 6