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Review

Pathogenicity and infection strategies of the fire blight pathogen *Erwinia amylovora* in *Rosaceae*: State of the art

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Plants are host to a large amount of pathogenic bacteria. Fire blight, caused by the bacterium *Erwinia amylovora*, is an important disease in *Rosaceae*. Pathogenicity of *E. amylovora* is greatly influenced by the production of exopolysaccharides, such as amylovoran, and the use of the type III secretion system, which enables bacteria to penetrate host tissue and cause disease. When infection takes place, plants have to rely on the ability of each cell to recognize the pathogen and the signals emanating from the infection site in order to generate several defence mechanisms. These mechanisms consist of physical barriers and the production of antimicrobial components, both in a preformed and an inducible manner. Inducible defence responses are activated upon the recognition of elicitor molecules by plant cell receptors, either derived from invading micro-organisms or from pathogen-induced degradation of plant tissue. This recognition event triggers a signal transduction cascade, leading to a range of defence responses [reactive oxygen species (ROS), plant hormones, secondary metabolites, ...] and redeployment of cellular energy in a fast, efficient and multiresponsive manner, which prevents further pathogen ingress. This review highlights the research that has been performed during recent years regarding this specific plant–pathogen interaction between *Erwinia amylovora* and *Rosaceae*, with a special emphasis on the pathogenicity and the infection strategy of *E. amylovora* and the possible defence mechanisms of the plant against this disease.

Introduction

Fire blight, caused by the Gram-negative bacterium *Erwinia amylovora*, represents an enormous threat to fruit cultivation in many parts of the world, as it can infect most plants of the *Rosaceae*, both ornamental and economic cultivars, among which apple and pear are important hosts. It is a complex disease which passes its entire cycle in close association with the host plant, where it is able to infect fruit, leaf, shoot and flower tissue. Infected plant parts will first appear water soaked, next they will turn dark green, wilt and finally turn brownish to black. In all cases, sticky, amber-like ooze drops, composed of viable bacteria in a polysaccharide matrix might be formed on the blighted plant parts. Fire blight is difficult to control, as it is able to rapidly spread in the plant and effective control methods still are lacking. Every year, infected apple and pear orchards are reduced in size because of quarantine-related measures. In Europe, fire blight is considered an increasing problem as higher temperatures, breeding of cultivars on susceptible rootstocks and the introduction of susceptible cultivars could enlarge the risk of infection in the near future (Deckers & Schoofs, 2008).

Abbreviations: Avr, avirulence; EPS, exopolysaccharides; JA, jasmonic acid; PR, pathogenesis-related; PRR, pattern recognition receptor; QS, quorum sensing; ROS, reactive oxygen species; SA, salicylic acid; T3SS, type III secretion system.

Pathogenicity and virulence of *E. amylovora*

Pathogenicity and virulence of the pathogen *E. amylovora* depend on different factors. Besides the production of the siderophore desferrioxamine for the acquisition of iron molecules from the host tissue (Dellagi *et al.*, 1998; Expert, 1999; Smits & Duffy, 2011) and the presence of other virulence factors such as metalloproteases (Zhang *et al.*, 1999), the presence of plasmids (Llop *et al.*, 2011, 2012; McGhee & Jones, 2000; Mohammadi, 2010), two-component signal transduction systems (Wang *et al.*, 2009; Zhao *et al.*, 2009) and histone-like proteins (Hildebrand *et al.*, 2006) are also important factors in pathogenesis. However, probably the most essential reasons for differences in virulence between different strains of *E. amylovora* are due to a variation in synthesis of exopolysaccharides and the mechanism of the type III secretion system (T3SS) and associated proteins.

Exopolysaccharides

Exopolysaccharides (EPS) have been suggested to play a key role in bypassing the plant defence system, in disturbing and obstructing the vascular system of the plant and in protecting the bacteria against water and nutrient loss during dry conditions (Denny, 1995; Ordax *et al.*, 2010).

One of these EPS is amylovoran, which is the main constituent of bacterial ooze. Amylovoran is a polymer of a pentasaccharide repeating unit that generally consists of four galactose residues and one glucuronic acid residue (Maes *et al.*, 2001; Nimtz *et al.*, 1996). The molecular size of amylovoran is influenced by several environmental conditions and cell-metabolism-related factors (Schollmeyer *et al.*, 2012). *E. amylovora* strains that do not have the capacity to produce amylovoran are non-pathogenic and are unable to spread in plant vessels (Bellemann & Geider, 1992). Another EPS that is synthesized by *E. amylovora* is levan. Lack of levan synthesis can result in a slow development of symptoms in the host plant (Geier & Geider, 1993).

The amylovoran synthesis (*ams*) gene cluster involved in the biosynthesis of amylovoran produces 12 *ams*-encoded gene products (AmsA to AmsL). AmsC, AmsH and AmsL are believed to be involved in oligosaccharide transport and assembly whereas AmsA possesses a tyrosine kinase activity. AmsB, AmsD, AmsE, AmsG, AmsJ and AmsK proteins appear to play part in annealing the different galactose, glucuronic acid and pyruvyl subunits to the lipid carrier in order to form an amylovoran unit. AmsF may process newly synthesized repeating units and/or be involved in their polymerization by adding them to an existing amylovoran chain. Finally, AmsI seems to have a distinct function in recycling of the diphosphorylated lipid carrier after release of the synthesized repeating unit (Bugert & Geider, 1997; Eastgate, 2000; Langlotz *et al.*, 2011).

Recently Koczan *et al.* (2009) discovered that EPS of *E. amylovora* are also involved in biofilm formation, which enables the bacteria to attach to several surfaces and each other (Koczan *et al.*, 2011; Ramey *et al.*, 2004). Studies of *Pseudomonas aeruginosa* have revealed that the process of biofilm formation can be divided into five distinct phases including reversible attachment, irreversible attachment, maturation 1, maturation 2 and detachment in the dispersion phase (Sauer *et al.*, 2002). Biofilm infections appear to be very persistent and it has been shown that bacteria that are able to compose a biofilm can be up to 1000-fold more resistant to antibiotic treatment than their planktonic counterparts (Gander & Gilbert, 1997). Koczan *et al.* (2009) have suggested that biofilm formation plays an important role in pathogenesis of *E. amylovora*, as their study showed that amylovoran is necessary for biofilm formation and that levan contributes to this biofilm formation. They also confirmed the results of Maes *et al.* (2001), in which it was shown that the quantity of amylovoran produced by individual *E. amylovora* strains is correlated with the degree of virulence.

The mechanistic details behind biofilm formation remain largely unknown but it is suggested that they are formed in response to environmental triggers (Davey & O'toole, 2000) and quorum sensing (QS) signals (Sauer *et al.*, 2002). Although research has shown that *Erwiniae* species produce two types of QS molecules namely *N*-acylhomoserine lactones and autoinducer-2 type signalling molecules (Barnard & Salmond, 2007; Molina *et al.*, 2005), Rezzonico & Duffy (2008) suggested a non-quorum-sensing role for the autoinducer-2 *luxS* gene due to a lack of genomic evidence for autoinducer-2 receptors.

Type III secretion system

Another important factor in pathogenicity is confined by the action of the T3SS. Gram-negative phytopathogenic bacteria such as *E. amylovora* utilize this evolutionarily conserved secretion system to export and deliver effector proteins into the cytosol of host plant cells through a pilus-like structure, which forms the central core element of the T3SS. The needle complex is composed of a large, cylindrically shaped macromolecular complex organized into a series of ring-like structures with inner rings, outer rings and a neck structure. It is embedded in the inner and outer membrane of the bacteria, while spanning the periplasmic membrane and extending into the extracellular environment with a needle filament (Alfano & Collmer, 2004; Block *et al.*, 2008; Büttner & Bonas, 2006; Büttner & He, 2009; Cornelis & Van Gijsegem, 2000; Galán & Wolf-Watz, 2006; Grant *et al.*, 2006; He *et al.*, 2004; Hueck, 1998; Jin *et al.*, 2001; Loquet *et al.*, 2012; McCann & Guttman, 2008; Mudgett, 2005; Schraidt & Marlovits, 2011).

The T3SS of plant-pathogenic bacteria is mainly made out of Hrc proteins, encoded by *hrp*-conserved (*hrc*) genes among plant-pathogenic bacteria and Hrp proteins, encoded by hypersensitive response and pathogenicity (*hrp*) genes. In *E. amylovora*, *hrc* and *hrp* genes are clustered in a pathogenicity island which contains four regions, i.e. a *hrp*/*hrc* region, a Hrc effectors and elicitors region, a Hrp-associated enzymes region and an island transfer region (Oh & Beer, 2005). The key regulatory gene is *hrp*L, which encodes the extracytoplasmic function of σ-factor HrpL, which in turn recognizes conserved sequence motifs (*hrp* boxes) located in promoters of *hrp* secretion genes and of genes encoding secreted proteins (McNally *et al.*, 2012; Oh *et al.*, 2005; Pester *et al.*, 2012).

To date, 12 proteins have been found that are secreted via this T3SS (Nissinen *et al.*, 2007). Four of them (Eop1, Eop3, Eop4 and DspA/E) have clear similarity to known effectors. The 200 kDa disease factor DspA/E for example, which is homologous to the type III effector AvrE discovered in soybean after inoculation with *Pseudomonas syringae* pv. *tomato*, is required for pathogenicity in several strains of *E. amylovora* and interacts with the intracellular domains of host plant receptor kinases and preferredoxin (Boureau *et al.*, 2006; Meng *et al.*, 2006; Nissinen *et al.*, 2007; Oh *et al.*, 2007, 2010; Triplett *et al.*, 2009). Efficient

secretion of DspA/E requires a type III chaperone DspB/F, which is a small acidic protein that binds to its cognate secreted protein (Gaudriault *et al.*, 2002; Triplett *et al.*, 2010). Five proteins belong to the helper protein class, namely Eop2, HrpK, HrpN, HrpW and HrpJ. Both Eop2 and HrpK have clear similarities to proteins in *P. syringae*, but their functions still remain elusive (Nissinen *et al.*, 2007). HrpN and HrpW instead are harpins. These proteins are glycine-rich, lack cysteine and are involved in inducing the hypersensitive response in non-host plants. Unlike HrpW, HrpN is required for full virulence in plants (Kim & Beer, 1998; Reboutier *et al.*, 2007; Sinn *et al.*, 2008; Wei *et al.*, 1992) and plays an important role in the translocation of DspA/E (Bocsanczy *et al.*, 2008). HrpJ has been postulated to act as an essential extracellular chaperone to prevent aggregation of harpins in the apoplast, and thus facilitate translocation of effector proteins into the host cells (Nissinen *et al.*, 2007). The three remaining proteins that are secreted via the T3SS are HrpA, TraF and FlgL. HrpA is an essential structural protein of the type III secretion pilus, TraF is involved in pilus formation and FlgL is similar to a flagellar hook-filament junction protein (Nissinen *et al.*, 2007).

Further signalling of the plant in response to *E. amylovora*

When a bacterial interaction with a plant occurs, there are intrinsically two levels of the plant immune system (Jones & Dangl, 2006; Robert-Seilaniantz *et al.*, 2007). The first level is performed by the action of multiple transmembrane pattern recognition receptors (PRRs) belonging to either the receptor-like kinase or receptor-like protein families. PRRs bear structural similarities to animal Toll-like receptors (He *et al.*, 2007; McDowell & Simon, 2008; Segonzac & Zipfel, 2011) and respond to multiple cell-surface components of Gram-negative bacteria, including lipopolysaccharide, a major constituent of the outer membrane (Dow *et al.*, 2000; Gerber *et al.*, 2004; Meyer *et al.*, 2001; Newman *et al.*, 2002), and flagellin, the protein subunit of the flagellum (Asai *et al.*, 2002; Takeuchi *et al.*, 2003). The second level of the plant immune system acts largely inside the cell, using the polymorphic nucleotide binding-leucine-rich repeats protein products encoded by plant-derived Resistance (R) genes to counter pathogen secreted effectors [Avirulence (Avr) proteins]. Avr proteins are considered factors that contribute to host infection, although the biochemical function of most Avr proteins remains unidentified. However, in those cases when Avr factors are recognized by resistant host plants through direct or indirect interaction with their complementary R-gene-encoded protein counterparts, they act as specific elicitors of plant defence rather than as virulence factors. When this genetic interaction takes place, a defence response is triggered and gene-for-gene resistance is established (Abramovitch & Martin, 2004; Belkhadir *et al.*, 2004; Bent & Mackey, 2007; Lahaye & Bonas, 2001; Mansfield, 2009; Martin *et al.*, 2003; McDowell & Simon, 2006; White *et al.*, 2000). Contrary to several other plant–bacteria interactions (Kunkel *et al.*, 1993; Ronald *et al.*, 1992; Tai *et al.*, 1999; Tsiamis *et al.*, 2000), until now no related avirulence gene and corresponding plant resistance gene have been reported in the pathosystem of *E. amylovora* and *Rosaceous* plants.

Nevertheless, much research has been performed concerning the signalling pathways *in planta* and the different modes of protection after inoculation with *E. amylovora*. Sarowar *et al.* (2011) for instance obtained a total of 3500 genes involved in metabolism, signal transduction and stress response, which were significantly modulated in fire-blight-infected blossoms of the apple cultivar 'Gala' and which indicates that several pathways are affected as a result of a successful infection with *E. amylovora*. Probably one of the earliest responses of a plant to fire blight is a rapid increase of reactive oxygen species (ROS), followed by the production of several secondary metabolites, plant hormones and components of other defence-related pathways, of which some are discussed here more in detail and are represented in Fig. 1.

ROS and the generation of an oxidative burst

ROS are normally only produced as side-products of some general pathways such as photosynthesis (Krieger-Liszkay, 2005). They are generated by various enzymic activities of which the best studied are NADPH oxidases. However, during an incompatible reaction, an increased production of ROS and a hypersensitive response can be observed. Interestingly, in the case of the compatible interaction between *E. amylovora* and a host plant, *E. amylovora* is perceived by this host plant as an incompatible pathogen, which results in the generation of ROS by the plant. These bursts of ROS seem to be paradoxically necessary for a successful bacterial colonization (Venisse *et al.*, 2001). The oxidative burst is elicited by HrpN proteins in non-host plants (Baker *et al.*, 1993; Chang & Nick, 2012; Desikan *et al.*, 1998; Livaja *et al.*, 2008) and by both HrpN and DspA in host plants (Venisse *et al.*, 2003). Furthermore, it is believed that the bacterial exopolysaccharides protect *E. amylovora* against the toxic effects of ROS since a non-capsular mutant of *E. amylovora* induced locally the same responses as the wild-type but was unable to further colonize the host plant (Venisse *et al.*, 2001).

The first detectable oxidants among these ROS produced are the molecules hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), which are commonly released into the apoplast of primarily infected plant cells (Baker & Orlandi, 1995; Foyer & Noctor, 2005; Torres *et al.*, 2006; Venisse *et al.*, 2001; Wojtaszek, 1997).

As a response of this increase in ROS, the concomitant activity of some antioxidative enzymes and redox metabolites is often reported in pear and apple, according to the necessity to carry out ROS detoxification to less toxic compounds (Faize *et al.*, 1999; Sklodowska *et al.*, 2011;

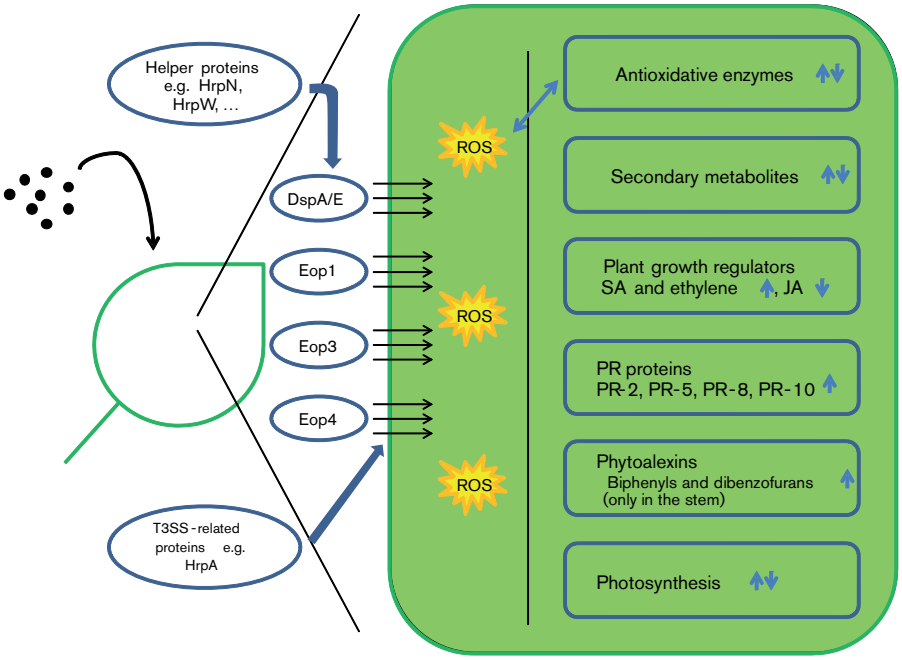

Fig. 1. Infection by *E. amylovora* causes the effector-related proteins DspA/E, Eop1, Eop3 and Eop4 to be secreted in the plant tissue, together with helper proteins and T3SS-related proteins. The result is an increase in ROS and different mechanisms to be triggered.

Venisse *et al.*, 2001, 2002, 2003; Viljevac *et al.*, 2009). Specific balances in antioxidative genes countering these ROS could explain why ontogenesis-related differences exist between cultivars regarding fire blight susceptibility (Vrancken *et al.*, 2012).

Secondary metabolites

Another phenomenon that could be observed as a result of a successful inoculation is the change in levels of phenylpropanoid–flavonoid pathway derived compounds with important groups such as flavonoids, phenolamines and lignin (Fischer *et al.*, 2007; Pfeiffer *et al.*, 2006; Treutter, 2001, 2010). The general phenylpropanoid metabolism generates an enormous array of secondary metabolites based on the few intermediates of the shikimate pathway as the core unit. The phenylpropanoid–flavonoid pathway is typified by an enormous complexity, which is caused by a large number of branches and branchpoints and many end products. Hence, the pathway has the ability to produce certain end products via different branches by combining the function of large superfamilies of reductases, oxygenases, ligases and transferases. The overall result is an organ- and developmentally specific pattern of metabolites, characteristic for each plant species.

Jensen *et al.* (2012) suggested that the expression of the phenylpropanoid pathway as a whole might be one of the many predictors of fire blight resistance. Burse *et al.* (2004) already showed that *E. amylovora* has the ability to protect itself against secondary metabolites in apple because of the internal efflux pump AcrAB, indicating the possible involvement of these metabolites in plant defence. Depending on the cultivar, the bacterial strain, the inoculation method and the time after inoculation, different results are reported throughout the literature regarding the phenylpropanoid–flavonoid pathway. For instance, both Venisse *et al.* (2002) and Milcevicova *et al.* (2010) found that most of the phenylpropanoid–flavonoid related enzymes investigated were repressed in some apple cultivars after inoculation with a specific fire blight strain, whereas Sklodowska *et al.* (2011) and Pontais *et al.* (2008) demonstrated that the level of some hydroxycinnamate derivatives was significantly augmented in both resistant and sensitive apple cultivars. Moreover, phloretin was found at a bacteriotoxic concentration in both genotypes, but *E. amylovora* exhibited the ability to stabilize this compound at sublethal levels (Pontais *et al.*, 2008). de Bernonville *et al.* (2011) proposed that the constitutive phenolic composition of two apple cultivars 'Evereste' and 'MM106' is not responsible for their contrasted differences in susceptibility to fire blight.

In pear, Gunen *et al.* (2005) reported a higher content of arbutin in resistant cultivars, while sensitive cultivars obtained a higher level of chlorogenic acid. Research done by our lab showed that the transcription patterns of two key genes, anthocyanidin reductase (*ANR*) and chalcone synthase (*CHS*), related to this phenylpropanoid–flavonoid pathway, considerately increased in *E. amylovora*-inoculated

mature leaves compared with the control and mock-inoculated mature leaves of the pear cultivar 'Conference', with the strongest reaction 48 h after inoculation (Vrancken *et al.*, 2012). These effects of *E. amylovora* were also visualized in histological sections, and confirmed by HPLC, as epicatechin, which is produced from cyanidin via *ANR*, increased 72 h after inoculation in *E. amylovora*-inoculated mature leaf tissue. The rise in *CHS* is in agreement with the work of Baldo *et al.* (2010) who used cDNA-amplified fragment length polymorphism analysis to demonstrate a sudden rise of *CHS* in the fire-blight-susceptible apple root stock M26 after inoculation with *E. amylovora*.

Although the real function of these secondary compounds in a fire blight–pome fruit interaction is still not clear, it has been reported many times in the literature that phenolic components have direct antioxidant properties which are even better than those of vitamins and ascorbic acid, for instance (Agati & Tattini, 2010; Feucht *et al.*, 1996; Gould, 2004). Moreover, they share the ability to influence cell signalling by downregulating pro-oxidant enzymes such as NADPH oxidases and lipoxygenases, by altering the phosphorylation state of target molecules or by chelating transition metals that mask pro-oxidant actions of reactive nitrogen and oxygen species, both in plants (Treutter, 2005) as in human and mammalian tissue (Fraga & Oteiza, 2011; Williams *et al.*, 2004). However, because of a lack of convincing spatiotemporal correlations with the flavonoid oxidation products, the widely accepted antioxidant function of flavonoids in plants is still a matter of debate (Hernández *et al.*, 2009). Furthermore, flavonoids have also been described as having antibacterial, antitoxin, antiviral and/or antifungal activities (Ardi *et al.*, 1998; Friedman, 2007; Treutter, 2005) or being involved in creating a structural defence, as research in other plant–pathogenic interactions revealed ultrastructural modifications with incorporated flavonoids, middle lamellae or callose-rich papillae to obstruct further progress of different pathogens (Dai *et al.*, 1996; Loureiro *et al.*, 2012; Nicaise *et al.*, 2009; Soylu, 2006). Probably, a combination of all these factors could affect susceptibility to *E. amylovora*.

Plant hormones

Jasmonic acid (JA), salicylic acid (SA) and ethylene are three distinct plant hormones which also interfere during microbial attack. Both the SA and JA defence pathways are mutually antagonistic (Chisholm *et al.*, 2006; Robert-Seilaniantz *et al.*, 2007), which has also been shown for the pathosystem *E. amylovora–Malus*.

Both de Bernonville *et al.* (2012) and Milcevicova *et al.* (2010) reported a significant accumulation of total SA in different apple cultivars after infection with *E. amylovora*; de Bernonville *et al.* (2012) also demonstrated a downregulation of JA levels in a susceptible cultivar of apple. Accordingly, treatment of these susceptible plants with methyljasmonate increases the resistance of these plants against *E. amylovora*, indicating that the downregulation of the JA pathway is a critical step in the infection process. Ethylene also seems to have a big part in the response of the plant after mechanical wounding and after pathogen attack. The group of Spinelli *et al.* (2011) measured ethylene production in both *E. amylovora*-inoculated and mock-inoculated apple plants, reaching a peak approximately five hours after inoculation. However, in mock-inoculated plants, this ethylene burst was much lower and faded away after six hours. Next to ethylene, the production of other volatiles such as 2,3-butanediole, isoprene-ozone and 3-hexenal were also detected in the *E. amylovora*-inoculated plants (Spinelli *et al.*, 2011). Whether this rise in ethylene and other volatiles is involved in a possible plant defence mechanism is still not known for this pathosystem.

Pathogenesis-related (PR) proteins

Pathogenesis-related proteins of plants are divided into more than 15 subfamilies and have been defined as host-originating proteins with direct antimicrobial activity that are induced only in response to a pathogen attack or related event. Induction of PR proteins has been found in many plant species belonging to different families, suggesting a general role of these proteins in adaptation to biotic stress conditions.

Not much scientific research has been published concerning PR proteins in woody fruit perennials after infection with *E. amylovora*. Only a systemic upregulation of the PR-5 (Bonasera *et al.*, 2006a; Venisse *et al.*, 2002), PR-2 (Bonasera *et al.*, 2006a; Heyens *et al.*, 2006), PR-8 (Bonasera *et al.*, 2006a) and PR-10 (Mayer *et al.*, 2011) families was reported in infected tissues. The *PR-1* gene family seems not to be involved (Bonasera *et al.*, 2006a; Pester *et al.*, 2012). Furthermore, overexpression of the *MpNPR1* gene confers activation of PR-2, PR-5 and PR-8 in *Malus* × *domestica* (Malnoy *et al.*, 2007). Although some of these PR proteins exhibit potential *in vitro* antimicrobial activities and their accumulation in the plant is related to plant resistance responses, a direct functional role in defence could not be demonstrated for all (Sels *et al.*, 2008; Van Loon & Van Strien, 1999).

Phytoalexins

Phytoalexins are low-molecular-mass secondary metabolites with antimicrobial activity, which are synthesized *de novo* after biotic and abiotic stress and occur in a wide variety of chemical structures and in different plant species. The biosynthesis of most phytoalexins, the regulatory networks involved in their induction by biotic and abiotic stress and the molecular mechanism behind their cytotoxicity remain largely unknown (Ahuja *et al.*, 2012; Chizzali & Beerhues, 2012). In both *Malus* × *domestica* 'Holsteiner Cox' and *Pyrus communis* 'Conference', the phytoalexin group of the biphenyls and dibenzofurans were

detected in the transition between healthy and diseased tissue of the stem after a fire blight infection. In leaves, no phytoalexins could be measured (Chizzali *et al.*, 2012a, 2012b; Hüttner *et al.*, 2010). Probably, both the outer-membrane protein TolC and the AcrAB transport system in *E. amylovora* play important roles as protein complexes that are capable in offering resistance to phytoalexins (Al-Karablieh *et al.*, 2009; Burse *et al.*, 2004).

Remarkably, the flavan-4-ol luteoforol, which is the unstable and highly reactive precursor of luteoliflavan, is induced in pome fruits after treatment with the growth regulator prohexiadone-Ca and shows phytoalexin-like properties against *E. amylovora* and other pathogens (Flachowsky *et al.*, 2012; Halbwirth *et al.*, 2003; Spinelli *et al.*, 2005).

Photosynthesis

Infection of apple by *E. amylovora* results in a decrease of photosynthetic activity, suggesting an inhibition of photosystem I and/or II (Bonasera *et al.*, 2006b). Similarly, changes in the chlorophyll fluorescence of *E. amylovora*-challenged apple leaves are observed prior to the development of disease symptoms. Both Heyens & Valcke (2006) and Baldo *et al.* (2010) noticed an induction of some photosynthetic genes during a *Malus–E. amylovora* interaction. Research by Singh *et al.* (2010) suggested that *FIBRILLIN4,* which is associated with photosystem II, could also play a part in fire blight infections, as the disease is more expressed in the knockdown mutant.

Conclusion

Despite the many efforts that have been put forward concerning the study of the pathogenicity and the infection strategy of *E. amylovora* and the possible defence mechanisms of the plant against this disease, this economically important pathosystem remains largely unexplored and/or is far from well understood. Little is known about end-stage disease, latent infections, survival away from the host, interaction with other microbial organisms and secondary bloom infections. Furthermore, regarding the infection process, the function and presence of avirulence genes, the amount of pathogenicity factors and the mechanism of the T3SS system remains poorly understood. The recent sequencing and annotation of the complete genome of *E. amylovora* CFBP1430 by Smits *et al.* (2010) is a welcome tool in revealing novel insights into the genome, which will surely lead to increased understanding of the virulence, host range and ecological behaviour of these bacteria on their host plants in the near future.

However, knowing the bacteria is not enough, as it is essential to study the plant as well. The availability of molecular markers and genetic mapping of fruit crops would allow identification of major resistance genes and disease-specific loci. An excellent review about candidate resistance genes and the application of genomics to improve fire blight management has been written by Malnoy *et al.* (2012).

In conclusion, gaining insight into infection strategies by *E. amylovora* and defence mechanisms of the host plant is crucial in obtaining a fire-blight-free environment.

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