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REVIEW PAPER

The importance of fungal pectinolytic enzymes in plant invasion, host adaptability and symptom type

Ph. Reignault · O. Valette-Collet · M. Boccara

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Abstract This review describes the extent to which recent works performed in the last decade have clarified the role of different pectinase activities in pathogenicity of fungi responsible for a wide array of diseases. Beyond physiological or biochemical investigations, strategies that have been used include gene cloning and expression studies as well as gene disruption or replacement. Pectinase involvement in fungal diseases has been considered according to the type of symptoms produced by the studied fungi. Although pectinolytic enzymes are clearly important for soft rot diseases, their role cannot be anticipated for other diseases as a whole. Each symptom type must therefore be investigated separately. Moreover, before antifungal strategies can be considered, several strains and/or mutants must be analysed.

Ph. Reignault (✉)
Mycologie-Phytopathologie-Environnement,
Université du Littoral Côte d'Opale, B.P. 699,
Calais Cedex 62228, France
e-mail: philippe.reignault@univ-littoral.fr

O. Valette-Collet
Pathologie Végétale UMR217,
Institut National Agronomique,
16 rue Claude Bernard,
Paris 75231, France

M. Boccara
Atelier de Bioinformatique,
Université Pierre et Marie Curie,
12 rue Cuvier,
Paris 75005, France

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Introduction

The most damaging pathogens of plants and crops are found within fungal parasites. They are responsible for worldwide yield losses that would be even more important without a successful control that still relies on the use of fungicides. Because of the growing concern about environmental and health damages caused by this control, the understanding of the mechanisms leading to fungal pathogenicity has become a classical and fundamental issue for plant pathologists, as well as a prerequisite to consider new control strategies. Whether they are root or leaf parasites, necrotrophic or biotrophic, fungi have to achieve at least partial degradation of the plant cell wall materials. Among them, pectin is a major component of the primary cell wall and middle lamella, and most fungal pathogens have therefore to degrade it at least partially when encountering plant cell physical barriers. Moreover, enzymatic degradation of pectin weakens the cell wall and exposes other cell wall components to other enzymes such as cellulases and hemicellulases. Pectin consists of so-called 'hairy' and 'smooth' regions. The hairy region known as rhamnogalacturonan is characterized by stretches of α-1-2-L-rhamnose-α-1-4-D-galacturonic acid dimers. The smooth region, or

homogalacturonan consists of a backbone of α-1-4 linked-D-galacturonic acid residues, partially methylated and possibly also acetylated (Carpita and McCann 2000).

To degrade pectin, fungi produce a battery of depolymerising enzymes. One possible way of degradation starts with pectin de-esterification into methanol and polygalacturonic acid (PGA) by pectin methylesterase (PME, E.C.1.1.11). PME activity is assumed to allow the subsequent action of depolymerising enzymes which, according to their mode of action, are classified into two groups: (1) those of *endo* cleavage mode, which is random and (2) those of *exo* cleavage mode, which act on the penultimate polymer bonds. Polygalacturonases (endo-PG, E.C.3.2.1.15 and exo-PG, E.C.4.2.1.67 and E.C.4.2.1.82) cleave glycosidic bonds by hydrolysis, whereas pectate lyases (endo-PL, E.C.4.2.2.2 and exo-PL E.C.4.2.2.9) break PGA into oligogalacturonides by β-elimination. In the alternative pectin degradation pathway, pectin lyase (PNL, E.C.4.2.2.10) is able to depolymerise native pectin via β-elimination into methylated galacturonide residues.

Although the first molecular study of the role of pectinolytic enzymes in fungal pathogenicity was reported more than 15 years ago (Scott-Craig et al. 1990), the first report of their importance has been long awaited (ten Have et al. 1998) and the status of pectinases regarding fungal pathogenicity is still an ongoing issue. Works corresponding specifically to fungal pathogens has been reviewed in the past (Cervone et al. 1996; Annis and Goodwin 1997; ten Have et al. 2002), but data reported so far almost exclusively refer to pathogenicity as a quantitative trait. However, as hypothesized by ten Have et al. (2002), the role of fungal cell wall degrading enzymes in pathogenesis may be related to the fungal lifestyle. In addition, previous studies (Reignault et al. 2000) have suggested that pectinases do not only determine the extent of plant invasion, but also the nature of the symptoms observed. Since then, several other experimental contributions have investigated the link between pectinases and pathogenicity for fungi involved in a wide array of diseases. This article aims to update previous reviews on pectinase importance in fungal pathogenicity and to present their role according to the main types of symptoms induced on plants, or plant organs, by fungi.

Fungal pectinolytic enzymes in soft rot diseases

Pectinase production and host adaptability Rotting of plant tissues is commonly accepted as the result of extensive degrading activity of pectinolytic enzymes on the plant cell wall components. Several studies have suggested in the past that differences in pectinase production for different strains of a given soft-rot pathogen account for differences in either host range or virulence, the latter being defined as the extent of rotting displayed on the host (Walton 1994). Here we shall briefly describe a few recent studies questioning pectinases and host adaptability. For example, studies of polygalacturonase (PG) production by *Didymella bryonia*, which causes black rot on the cantaloupe fruit (*Cucumis melo* var. *cantaloupensis*), showed a positive correlation between the lesion size and the total fungal PG activity measured in the decayed tissues. Since no PG activity could be detected in healthy fruit, PGs detected in decayed cantaloupe fruit were considered as exclusively being of fungal origin. The measured correlation suggests that PGs play an important role in *D. bryoniae* rotting activity during cantaloupe fruit decay (Zhang et al. 1999). Using an isolate of the same fungus that produces both PNL and PME activities but no PG, Chilosi and Magro (1998) measured the activities produced in diseased melon hypocotyl tissues. They observed that PNL activity was highly correlated with the disease severity and concluded that PNL was the main pectinase activity associated with rotting in melon seedling hypocotyls. Pectinolytic enzymes of *D. bryoniae* are therefore involved in tissue maceration occurring during soft rot, but the determining activity could be either PG or PNL, depending on the infected organ or plant. The same authors quantified spectrophotometrically and analyzed on isoelectric focusing (IEF) gels an *in vivo* sequential production of PNL and PG from *Botrytis cinerea* (Chilosi and Magro 1997). They used different types of fungal inoculum (ungerminated and germinated conidia, mycelium) on *B. cinerea*/soybean, *B. cinerea*/apple fruit and *B. cinerea*/zucchini fruits interactions. Whereas in zucchini and soybean, both PG and PNL were both produced during fungal colonization, in apple fruit, only two PGs were associated with maceration. On *B. cinerea*/apple fruit combination, Reignault et al. (2000) showed that types of maceration varied, depending upon PG isozyme patterns. No endo-PG

activity was found in apples infected by wild-type strains causing so-called firm-rotting whereas two neutral endo-PG isozymes were secreted by wild-type strains causing typical soft-rot symptoms. Since no difference was observed in the colonization level, these results suggest that these *B. cinerea* PGs determine the qualitative component of apple fruit rotting (firm or soft rotting) but do not interfere with the quantitative one (colonization extent). In these two studies, PG isozymes patterns were obtained from apple tissues infected with *B. cinerea* and showed partial similarities. Among the two neutral PGs observed by Reignault et al. (2000) on apple soft rot symptoms, one of them is either one of those described on infected apple tissue by Chilosi and Magro (1997) or an isoform very closely related to it.

At the molecular level, *B. cinerea* possesses a large family of genes encoding endo-PGs (Wubben et al. 1999). The expression of a set of six distinct PG-encoding genes (*Bcpg 1–6*) was investigated by ten Have et al. (2001) on four different host plants: tomato, broad bean, apple and zucchini. The pattern of expression of these *Bcpg* genes showed that they are differentially expressed, depending again on the infected host. Whereas *Bcpg1* was expressed early in all the tested tissues, *Bcpg3* and *Bcpg5* were expressed in apple tissue only. On the other hand, *Bcpg2* expression was also found during the early infection of all plants, except in apple fruit were it could not be detected. In *Sclerotinia sclerotiorum*, another typical soft rot plant pathogen, at least 16 different isoforms are sequentially produced during the *in vitro* growth of the fungus (Martel et al. 1998). A set of four endoPG-encoding genes, *sspg1*, *sspg3*, *sspg5* and *sspg6*, and two exoPG-encoding ones, *ssxpg1* and *ssxpg2*, was also isolated. The *sspg1* gene could be associated with pathogenicity since it has been shown to be highly expressed in pathogenic conditions on *Brassica napus* (Li et al. 2004). Another study of *in planta* expression of some of these genes showed the *sspg1* was expressed earlier than *sspg3*, *5* and *6* during infection (Kasza et al. 2004). It could be hypothesized that particular PG isoforms are essential not only for early pathogenicity stages, but also for the induction of a second set of cell wall degrading enzymes.

Oomycetes from the genus *Phytophthora,* which have a fungus-like biology but distinct phylogenic affinities, also produce PG activities. Their evolution towards the acquisition of genes encoding for activities shared with phytopathogenic true fungi could have been required for their adaptation to plant host invasion and rotting. Indeed, phylogenetic analysis of the PG-encoding sequence from *Phytophthora cinnamomi* revealed a surprisingly closer relationship with PG-encoding sequences from phytopathogenic true fungi than with those from plants (Götesson et al. 2002). Possible similar roles in pathogenicity may be investigated in the coming future. A PG-encoding gene has been cloned from *Phytophthora parasitica*, responsible for root and fruit rot on a wide variety of crops. This gene is highly expressed during the interaction with tomato, suggesting its involvement in infection (Yan and Liou 2005). This recent work confirmed that soft rotting fungi are equipped with an enzymatic pectin degradation battery that is likely to explain the great host range and adaptability of these fungi. However, such evidence is not so conclusive when considering the importance of pectinolytic enzymes in pathogenicity. Construction of mutants in the corresponding encoding genes and evaluation of their pathogenicity therefore appeared to be necessary.

Disruption of pectinase genes It has been thought for a long time that PME activity was important for the subsequent action of depolymerizing enzymes. A disrupted mutant was obtained in the *Bcpme1* PME-encoding gene from the *B. cinerea* Bd90 wild-type strain. *In vitro* growth of the mutant is similar to that of the wild-type on glucose medium, whereas it is reduced on a pectin medium. Pathogenicity tests showed a reduced rotting activity of the mutant on *Arabidopsis thaliana*, grapevine (*Vitis vinifera*) leaves and apple fruit (*Malus domestica*). Moreover, PME activity is 75% lower in the mutant than in the wild-type, and all these data showed that PME activity encoded by *Bcpme1* is a key virulence factor in *B. cinerea* pathogenicity (Valette-Collet et al. 2003). However, Kars et al. (2005b) obtained via PCR-based targeted mutagenesis, mutants lacking both *Bcpme1* and *Bcpme2* genes from a distinct B05.10 wild-type strain. Such mutants were not affected either in their *in vitro* growth on a pectin-rich medium or their pathogenicity on tomato and grapevine leaves. As mentioned by Kars et al. (2005b), PME is likely to act in a strain-specific manner in *B. cinerea*, as it has been reported in the case of another virulence factor, the botrydial toxin (Siewers et al. 2005). A *B. cinerea*

disrupted mutant in the *Bcpg1* gene remains pathogenic on tomato fruits and leaves and apple fruits. However, a significant decrease in lesion growth beyond the inoculation spot is observed on all three inoculated host tissues (ten Have et al. 1998). These results indicate that the *Bcpg1* gene might be partially required for virulence of *B. cinerea*. According to *in planta* endo-PG gene expression patterns mentioned above, a *Bcpg2* mutant can be expected to exhibit a reduced rotting ability on broad bean (*Vicia faba*) but not on apple (*Malus domestica*), whereas a *Bcpg3* mutant could be predicted to show the opposite pathogenicity potential (ten Have et al. 2001, 2002). The molecular deletion of the *Bcpg2* gene was performed and the macerating activity of the corresponding mutant was indeed shown to be reduced by 50–85% on both tomato and broad bean, indicating that BcPG2 is important for *B. cinerea* pathogenicity (Kars et al. 2005a).

A single PG in *Aspergillus flavus* was shown to determine most of the fungal aggressiveness on cotton bolls (Shieh et al. 1997). Indeed, *A. flavus* isolates can be divided into PG-producing and non-producing groups. The PG-producing group is more pathogenic, since it induces more damage and colonizes cotton bolls to a greater extent than the group that does not produce PG. The addition of the *pecA* PG-encoding gene to a non-producing strain resulted in more damage to the intercarpellary membrane and to greater fungal colonization of the adjacent locule. In the same way, the targeted disruption of *pecA* in a PG-producing strain significantly reduces aggressiveness. *Alternaria citri*, which causes post-harvest black rot on many *Citrus* cultivars, produces *in vitro* in a pectin-containing medium a single endo-PG. The PG-encoding *Acpg1* gene was cloned and the corresponding disrupted mutant was obtained. Its *in vitro* growth on the pectin medium is reduced by 42% compared to the wild-type growth. The *A. citri* wild-type is able to grow on the peel of several grapefruit cultivars and cause black rot, whereas the *Acpg1* disrupted mutant is not (Isshiki et al. 2001). In *Nectria haematococca* (*Fusarium solani* f. sp. *pisi*), which is responsible for fusariose and soft rot on pea, the disruption of one of the PL-encoding *pelA* or *pelD* genes caused no detectable effect. However, the double disruption of both *pelA* and *pelD* genes drastically reduced the extent of induced symptoms (Rogers et al. 2000). *Colletotrichum gloeosporioides*, the causal agent of anthracnose disease and fruit rotting in a wide variety of temperate and subtropical crops, produces a wide range of pectinase activities during host tissue colonization: endo-PG, pectin lyase, pectin methylesterase and pectate lyase (Yakoby et al. 2000). When the *pelB* gene of *C. gloeosporioides* is disrupted, the corresponding mutant exhibits only a 15% *in vitro* growth reduction on a pectin-containing medium. However, when the mutant is inoculated onto avocado fruits, a 36 to 45% reduction in estimated rotting diameter is observed compared with the wild-type and the undisrupted transformed isolate controls (Yakoby et al. 2001). Therefore, although not being of major importance *in vitro*, the corresponding PLB enzyme contributes significantly to *C. gloeosporioides* pathogenicity on avocado fruit.

Biological activities of purified pectinases from macerating fungi One of the most recent and unexpected findings was that pectinases purified from rotting fungi could be associated with other symptomatic features, such as necrosis or necrosis-associated cell death. Among five isozymes expressed by *B. cinerea* in the yeast *Pichia pastoris*, two purified endo-PG enzymes, BcPG1 and BcPG2, exhibited a strong necrotising activity on tomato, broad bean and *A. thaliana*. The deletion of the *Bcpg2* gene resulted in a strong reduction in fungal pathogenicity on tomato and broad bean. Moreover, the *Bcpg2* mutant both delayed the appearance of primary necrotic lesions and reduced by 50% the expansion of subsequent maceration, suggesting that the macerating ability of the fungus is at least partially conditioned by the necrotising activity of pectinases (Kars et al. 2005a). In addition, a recent study identified BcPG1, a glycoprotein from *B. cinerea* strain T4 with a PG activity similar to that of BcPG1 described above, as an elicitor of defence reactions. These reactions included the production of active oxygen species, defence gene transcript accumulation and phytoalexin production (Poinssot et al. 2003), that are all known to be associated with cell death. These authors showed that the eliciting activity was due not only to the release of oligogalacturonates but also to the recognition of the protein itself. BcPG1 is therefore involved in two antithetic functions: aggressiveness and elicitation of plant defences. A similar example is provided by a basic endo-PG isoform produced by *S. sclerotiorum*. This isoform, when applied to soybean cells, induced a programmed cell

death, altogether with a cytosolic Ca^{2+} elevation, a cytochrome c release and a caspase-like protease associated it (Zuppini et al. 2005).

These recent studies confirmed the role of fungal PGs in soft rot symptoms, and increased their importance to other components of the interaction, such as necrosis ability and the induction of plant defence responses. The activation in infected plants of necrotising defences such as an oxidative burst might facilitate infection by necrotrophic fungi, while rendering the infection site inappropriate to the development of biotrophic fungi.

Pectinolytic enzymes from non-macerating fungi

Wilt pathogens Plant pathogenic fungi responsible for wilt symptoms produce pectinases which may also be of particular importance since infected host xylem vessels are occluded by pectic gels originating from exposed middle lamellae of pit membranes and perforation plates. Gel formation reduces vascular flow, causes water stress and eventually the wilting of the plant (Ouellette et al. 1999). *Fusarium oxysporum* f. sp. *dianthi* attack on carnation (*Dianthus caryophyllus*) is characterized by a severe degradation of vascular tissues, hollowed stems and wilted leaves. The role of PG and related cell wall degrading enzymes such as PME in the wilting process was investigated. The amount of PG activity was found to be highly correlated to the disease development on carnation cultivars expressing different levels of resistance to *F. oxysporum* f. sp. *dianthi* (Baayen et al. 1997). However, more recent molecular studies on a distinct pathosystem seem to contradict such a correlation. The disruption of the *pgx4* gene from *Fusarium oxysporum* f. sp. *lycopersici*, which encodes an *in planta* expressed exo-PG has no effect on virulence towards tomato (Garcia-Maceira et al. 2000). The same lack of importance was observed after the inactivation of *pg5*, which encodes an extracellular endo-PG expressed in tomato roots during the initial stages of infection (Garcia-Maceira et al. 2001). Similarly, in the same fungus, inactivation of the *pg1* gene, which encodes the major endo-PG isozyme, had no effect on muskmelon plant infection (Di Pietro and Roncero 1998).

Plant wilting can also be caused by an infection with *Fusarium moniliforme* and the symptoms caused by this fungus cannot always be distinguished from those caused by the previously described *F. oxysporum*. However, in this case, wilting is caused by root necrosis or root rotting rather than by vessel occlusion. A site-directed mutagenesis of three amino acid residues located at the putative active site of the PG from *F. moniliforme* has been performed. A single mutation in the histidine residue 234 was critical for fungal macerating activity on potato medullary tissues (Caprari et al. 1996).

It seems therefore that in fungal pathogens causing wilt diseases associated with xylem vessel occlusion, the disruption of a single pectinase gene has no effect on pathogenicity, even for genes encoding major or *in planta* strongly expressed activities. However, pectinases are likely to determine wilting when it is a consequence of initial tissue necrosis or maceration. Further studies should aid in the confirmation of these results.

Fungi causing leaf spot diseases *Cochliobolus carbonum* is the causal agent of Northern leaf spot on corn. It mainly attacks foliar tissues, but also the stalks and ears of susceptible maize cultivars. Scott-Craig et al. (1998) disrupted the *pgx1* gene encoding an exo-PG in *C. carbonum*. The *in vitro* growth of a *pgx1* mutant is reduced by 20% on pectin medium but it remains pathogenic. A double mutant, disrupted in *pgx1* but also in *pgn1*, which encodes an endo-PG activity, grows to the same extent as the *pgx1* single mutant on pectin medium and also remains pathogenic, although it exhibits less than 1% of total wild-type PG activity. The fact that *pgx1* and *pgn1* genes are not essential for *C. carbonum* virulence on maize could be explained by the fact that pectin content level is lower in Monocots such as maize than in Dicots. Alternatively, pectinases may be important only for the saprophytic phase of the fungal life. However, additional pectinase activites that would be expressed *in planta* should be investigated in the future for their importance in pathogenicity.

Two distinct *Alternaria* species are known to be pathogenic on *Citrus* sp.: *Alternaria citri*, which is the causal agent of black rot, and *Alternaria alternata* strain AC325, which causes brown spot on a narrow range of *Citrus* species, e.g. rough lemon and rangpur lime. The necrotic brown spots are surrounded by a chlorotic halo on young leaves as the result of host-selective ACR-toxin production. Both species are

morphologically similar and the endo-PGs produced by these fungi have similar biochemical properties. Moreover, the PG1-encoding *Aapg1* gene of *A. alternata* showed 99.6 and 99.2% identity at nucleotidic and amino acid levels, respectively, to the *Acpg1* gene of *A. citri*. However, the phenotypes of the two *pg1* mutants were very different depending on whether it occurred in *A. citri* or in *A. alternata*. An *Acpg1* mutant of *A. citri* is significantly reduced in its ability to cause black rot symptoms on citrus whereas the *Aapg1*-disrupted mutant grows as well as the wild-type on medium containing pectin or glucose and is still fully pathogenic on rough lemon leaves. These results clearly show that PG1 is not essential for brown spot symptoms caused by *A. alternata* and that the role of pectinases may vary greatly with the disease type within the *Alternaria* genus (Isshiki et al. 2001). These two recent studies failed to include pectinolytic enzymes among the major factors of leaf spot diseases.

Biotrophic and hemibiotrophic pathogens It is assumed that, unlike necrotrophic plant–fungal relationships where extensive cell wall degradation is associated with fungal progression, in biotrophic and hemibiotrophic relationships, pectin degradation must be under tighter regulatory control in order to minimize host cell wall damage (ten Have et al. 2002).

Concerning hemibiotrophic fungal pathogens, correlations between pathogenicity and the *in vitro* production of pectin degrading enzymes were investigated in *Mycosphaerella graminicola*, responsible for wheat leaf blotch. Correlation tests and principal component analysis showed that PG activity was positively correlated with three distinct pathogenicity components: the time when 50% of the leaves contained a lesion, the time when 50% of the leaves showed sporulation, and the lesion frequency (Douaiher et al. 2007). In addition, as mentioned above for macerating fungi, pectinases from hemibiotrophs also possess biological activities. The elicitor activity of the CLPG1 polygalacturonase from *Colletotrichum lindemuthianum* has been established by Boudart et al. (2003). This activity led to the induction in tobacco of PR-proteins such as glucanase and of reactive oxygen species metabolism, and induced a leaf necrosis. In contrast to the work done on *B. cinerea* PG which could have an eliciting activity independent of its enzymatic activity (Poinssot et al. 2003), a functional catalytic site of the enzyme was necessary for the elicitor activity of CLPG1.

Cytological data showed that fungal extracellular pectinolytic enzymes were acting *in planta* and resulted in host wall alterations during infection by biotrophic fungal parasites. *Claviceps purpurea* is a biotrophic and organ-specific fungus. It attacks young ovaries of cereals and grasses and is responsible for ergot. Immunocytology using a JIM monoclonal antibody specific for non methyl-esterified epitopes of pectin demonstrated the presence of PGA in rye ovaries. During late phases of infection by *C. purpurea*, the absence of any JIM label emphasized the complete degradation of PGA.

In addition to these cytological data, a molecular investigation showed that two endo-PGs-encoding genes, *cpg1* and *cpg2*, were expressed during various stages of rye infection (Tenberge et al. 1996). The pectinase-encoding gene expression was also investigated in conidia and germlings of the obligate parasite *Blumeria graminis* f. sp. *tritici* (Suzuki et al. 1999). Two partial clones of the PG-encoding genes *pg1* and *pg2*, the PL-encoding gene *pel1* and the two PNL-encoding genes *pnl1* and *pnl2* were obtained: *pg1*, *pg2*, and *pnl2* were all expressed in ungerminated conidia and during pathogenesis while *pnl1* and *pel1* were only expressed *in planta*. Two PNL-encoding genes, *pnl-1* and *pnl-2*, were cloned from *C. gloeosporioides* f.sp. *malvae*, the hemibiotrophic agent of anthracnose, and *pnl-1* was shown to encode a cellulose-binding domain. These two PNL-encoding genes showed distinct expression patterns: in contrast to *pnl-2*, *pnl-1* was shown to be expressed during the establishment of the necrotrophic phase of the infection. The differences of these two isoforms may be linked to the fungal growth ability on different host tissues and during the biotrophic and necrotrophic phases of infection (Wei et al. 2002). The pectinolytic enzyme production was also studied at the biochemical level during corn smut development in two strains of *Ustilago maydis* differing in their pathogenicity (Cano-Canchola et al. 2000). Chlorosis and production of teliospores was coupled with an increase in PL activity 3–4 days after inoculation while anthocyanin production and formation of galls was associated with PG production 7–8 days after inoculation.

Only few disruption studies have been performed for biotrophic fungi. *Claviceps purpurea* mutants

lacking both *cpg1* and *cpg2* genes are not affected in their *in vitro* growth characteristics, but they are nearly non-pathogenic on rye. The endo-PGs encoded by *cpg1* and *cpg2* therefore represent essential pathogenicity factors in the interaction system *C. purpurea*/*Secale cereale* (Oeser et al. 2002).

Further investigations, particularly gene disruptions, are still necessary to confirm the role of pectinases at a precise time and location during plant biotrophic or hemibiotrophic fungal interactions. A perspective in that field can be given by the application of the biolistic transformation method of *B. graminis* to mutant construction in other obligate parasites that are often difficult to transform (Chaure et al. 2000).

Fungal pectinolytic enzymes in wood-rot diseases

Studies of the plant wall degradation process in woody tissues by rotting fungi generally focus on lignin and cellulose degradation, with little attention paid to alteration of pectin. Moreover, molecular studies regarding fungi causing wood-rot disease have remained limited since these pathogens are mostly basidiomycetes, for which genetic transformation still needs improvements.

The survey of PG production by fungi responsible for wood decay led Green and Clausen (1999) to the conclusion that most brown- and white-rot fungi (12 out of 15 and 6 out of 8 isolates of different species, respectively) have the enzymatic capacity to hydrolyse PGA through endo-PG activity. However, wood core colonization by white-rot fungi resulted at the microscopic level in hydrolysis of bordered pit membranes whereas brown-rot fungi only induced torus damage with a weakening and a tearing of the pit membranes, suggesting that distinct types of PG activities are involved. *Heterobasidion annosum* is a basidiomycete that causes root and butt rot and extensive mortality to many forest trees. Its population consists of three distinct intersterile groups (IG), called P, S and F. The different IGs have both different host ranges and morphological characteristics: IG-P strains were detected in the pectin-rich cambial zone of pine trees, where IG-S strains were not observed (Comparini et al. 2000). These authors also compared the multiple isoforms of PG, PNL, PME produced by the three IG groups of *H. annosum* by IEF-PAGE analysis. IG-S is characterized by a narrow host range, and produces weak pectinolytic activities, while IG-P and IG-F produces higher levels and has a wider host range, suggesting a correlation between PG level and *H. annosum* adaptability to different plant host tissues.

As far as we know, only one pectinase gene disruption experiment has been conducted on a fungal wood pathogen. The chestnut blight fungus *Cryphonectria parasitica* induces canker formation on American chestnut. It penetrates through a pre-existing wound of lignified zones and its penetration is followed by the formation of a defence zone by the wounded epiderm. Its also leads to the destruction of the host vascular cambium tissues and to the death of distal parts of the chestnut tree, both suggesting the involvement of cell wall degrading enzymes. The gene *enpg-1*, encoding the major *in vitro* extracellular endo-PG of *C. parasitica* was disrupted and no reduction in canker formation on dormant American chestnut stems was observed. The level of PG activity measured in canker bark tissues infected with the *enpg-1* mutant was indistinguishable from that found in canker tissues infected with the virulent strain. The identification of two acidic PG activities expressed predominantly *in planta* provides new opportunities for examining the importance of PGs in *C. parasitica* pathogenesis (Gao et al. 1996).

Conclusion

Cell-wall degrading enzymes, and among them pectinases, are considered as typical examples of ‘basic’ compatibility factors, meaning that they are thought to be required for pathogenicity but not to determine any host or cultivar specificity (Reignault and Sancholle 2001). However, the range of activities involved in pectin degradation and operating through different degradation pathways (for instance hydrolysis or β-elimination) may lead to a greater fungal adaptability on distinct host plant tissues, that may differ in pectin structure. The importance of several pectinases has been established for soft-rotting fungi in several pathosystems. The role of pectinolytic enzymes is clearly more important in the case of soft-rotting and macerating fungi than in other plant–fungus interactions. However, the effects of pectinase-encoding gene disruption also appear to vary according to the number of such genes expressed in the fungal

genome and therefore to the number of corresponding isozymes. Disruption of single copy genes – such as PME-encoding genes in *B. cinerea* – reduces fungal ability to macerate plant tissues. However, when considering a fungus possessing multiple genes encoding pectinolytic enzymes – like *B. cinerea* PG-encoding genes – some enzymes may prove to be more important than others depending on several parameters such as host plant, infection phase or symptom type. Future studies should test the actual contribution to pathogenicity of any second set of additional pectinases produced in the mutants in order to investigate some compensatory mechanisms. Studies leading to conflicting conclusions show that in order to validate virulence factors by targeted mutagenesis in future studies, several strains should be used. Moreover, the recent progress in functional and comparative genomic studies in plant pathogenic fungi should provide in the future useful information about genes involved in pectin degradation (Xu et al. 2004). An alternative strategy to disruption of pectinase encoding genes would be the identification and mutation of the corresponding regulatory genes. Transcriptional regulation of fungal pectinases has been reviewed elsewhere (Aro et al. 2005). For example, targeted gene replacement in *Cochliobolus carbonum* of the *SNF1* gene led to mutants that were strongly down-regulated for their pectinase and other cell wall degrading enzyme mRNA production and exhibited impaired pathogenicity on maize (Tonukari et al. 2000).

As shown with several examples presented above, the type of induced symptoms, besides the quantitative extent of pathogenicity, is at least partially determined by the expression of these activities. However, pectinases such as PGs from macerating fungi, beyond being active defence inducers in plants, have been shown to induce necrosis instead of maceration, as would have been expected by comparison with bacterial pathogens (Collmer and Keen 1986; Shevchik et al. 1998). For wilt and spot diseases or fungi attacking trees, a role in a precise step of infection or importance depending on tissue localization during infection, cannot be ruled out and is even sometimes suggested at the current stage of our knowledge. In addition, it would be interesting to investigate the importance of pectinases in mycorrhizal symbiosis. As in other biotrophic interactions, a tightly regulated production of pectinases during the establishment of the fungus–root interface in mycorrhizae may facilitate non-destructive fungal invasion of host cells. Their possible importance in ectomycorrhizal symbiosis has been reviewed in the past (Cairney and Burke 1994). Since then, correlative and biochemical studies performed so far suggest that pectinases play a role in degrading pectin during the establishment of such a symbiosis (Perroto et al. 1997). Transformation of mycorrhizal fungi is still difficult to achieve (Saito et al. 2001; Tagu et al. 2002) and no recent molecular studies on pectinolytic enzymes produced by mycorrhizal fungi are yet available.

Finally, there are several recent studies aimed at using the knowledge that has been recently obtained about the importance of pectinases for anti-fungal strategies. As a response to the fungal secretion of PGs, plants produce polygalacturonase-inhibiting proteins (PGIPs) as a defence against PG-mediated damage. The inhibiting activity of PGIPs directly reduces the aggressive potential of PGs and consequently limits fungal invasion. In addition, PGIPs cause PGs to form oligogalacturonides consisting of 7–15 units that induce defence responses (De Lorenzo and Ferrari 2002). Experiments involving transgenic plants have demonstrated that constitutively expressed PGIPs are active against pathogens infections. Transgenic expression of pear PGIPs in tomato slowed the expansion of disease lesions and associated tissue maceration by *B. cinerea* (Powell et al. 2000). Similarly, over-expression of the two functional PG inhibitors AtPGIP1 and AtPGIP2 in *Arabidopsis* significantly reduced *B. cinerea* disease symptoms (Ferrari et al. 2003). Another reason for a PGIP-based antifungal strategy in plants is provided by the antisense expression of the *AtPGIP1* gene in *Arabidopsis* that made such plants exhibit an increased susceptibility to infection by *Botrytis cinerea* (Ferrari et al. 2006). In pathosystems for which polygalacturonases determine pathogenicity, overproduction of a PGIP via plant transformation or breeding selection could thus prove a way to counteract fungal infections (Di Matteo et al. 2006; Di et al. 2006). However, PGIP must be produced before symptom appearance.

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