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Mini review

Pectin-degrading enzymes and plant-parasite interactions

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Abbreviations: DP = degree of depolymerization; KDG = 2-keto-3-deoxygluconate; PGIP = polygalacturonase-inhibiting protein; PG = polygalacturonase; PL = pectate lyase; PME = pectin methylesterase; PNL = pectin lyase; *pel*, *peh*, *pem* = gene symbol of pectate lyase, polygalacturonase and pectin methylesterase respectively

Introduction

The first contact of microbial plant parasites with their hosts occurs at the plant surface. During penetration and colonisation, they conflict with the cell walls of the host. Breakdown of plant cell wall is essential not only for parasite spreading in tissue, but also for release of assimilable carbohydrates, or accessibility to protoplast and cell killing. Biochemical analysis of the infection process has demonstrated that microbial plant pathogens produce a set of depolymerases capable of attacking the different carbohydrate polymers and proteins composing the plant cell wall. A crucial role in pathogenesis, however, has been demonstrated only for the pectin-degrading enzymes. These are the first class of enzymes produced during plant infection, accounting for the rapid and extensive degradation of cell wall and cell death, and reproduce the major symptoms of diseases caused by many necrotrophic pathogens, particularly those which produce soft-rot diseases.

The traditional approach to studying pectin-degrading enzymes is through their extraction from infected tissue, purification, characterisation and assessment of their ability to degrade purified plant cell wall or macerate plant tissues. Recent advances in gene manipulation of micro-organisms has provided powerful tools for understanding the contribution of pectic enzymes to the infection process. This review focuses on some aspects of pectic enzymes on which recent insights have been gained: the regulation of expression of pectic enzymes in bacterial and fungal parasites and their role in pathogenesis and host defence reactions.

Regulation of pectic enzyme production and pathogenesis

Regulation of depolymerases' production and pathogenesis are two strictly linked processes. Against the complexity of plant cell wall polymers [Carpita and Gibeaut, 1993], the pathogen must have an efficient and suitable set of depolymerising enzymes and isoenzymes. Sequence of production, speed of synthesis, concentration and diffusion of enzyme molecules around the site of secretion are fundamental aspects of the pathogenetic process and vary with the nutritional and ecological requirements of the pathogen. To finely control these processes, the pathogen has developed a sensor apparatus to perceive the characteristics and the modifications of the cell wall environment, and a regulatory system able to integrate the various stimuli and to control and coordinate the production of the most effective cell wall degrading-enzymes. How this articulated and interactive mechanism functions is becoming less obscure since some enzyme and regulatory genes have been cloned and manipulated.

Regarding pectic enzymes (which include endo- and exo-PG, endo- and exo-

PNL and PME), genes have been cloned and characterised from several bacterial [Collmer *et al.*, 1985; Dow *et al.*, 1989; Hinton *et al.*, 1990; Liao, 1991; Liao *et al.*, 1992; Nikaidou *et al.*, 1993; Rodriguez-Palenzuela *et al.*, 1991; Schell *et al.*, 1988] and fungal [Bussink *et al.*, 1991a, b; Caprari *et al.*, 1993; Dean and Timberlake, 1989; Gonzales-Candelas and Kolattukudy, 1992; Scott-Craig *et al.*, 1990] plant pathogens.

Extracellular production of pectinases and, in general, of most extracellular depolymerases, is the result of activation of synthesis and secretion. As a general mechanism, pectic enzyme production is subjected to induction by specific compounds structurally related to the substrate and to catabolic repression by readily metabolised carbohydrates (e.g. glucose) or products released from the substrate at a rate exceeding the utilisation ability of the cell (self-catabolite repression) [for review see Cooper, 1983]. In the absence of inducing substrate, enzyme activity may derive from constitutive synthesis, or from basal synthesis of inducible enzymes. A basal level of activity is fundamental in the early phase of infection to solubilize cell wall components that enter the catabolic cascade triggering inducible enzyme synthesis.

Pectic enzyme production has been extensively studied in prokaryotic micro-organisms, particularly in soft rot Erwinias. In *Erwinia chrysanthemi*, studies using mutants have shown that the true inducers of the PL synthesis are intermediates of the pectate catabolic pathway, mainly KDG or its derivates [Condemine *et al.*, 1986; Hugouvieux-Cotte-Pattat and Robert-Baudouy, 1987; Nasser *et al.*, 1991]. On the other hand, pectate is only marginally effective in PL synthesis by *Erwinia carotovora* [Chatterjee *et al.*, 1991]. In most *Erwinia* spp., PNL production is induced by DNA-injuring agents [Itoh *et al.*, 1980 and 1982; Tomizawa and Takahashi 1971; Tsuyumu and Chatterjee, 1984]. Other factors, like temperature [Perombelon and Kelman, 1980], oxygen limitation, osmolarity of the culture medium and nitrogen starvation [Hugouvieux-Cotte-Pattat *et al.*, 1992], iron concentration [Sauvage *et al.*, 1990] and Ca^{2+} [Liao *et al.*, 1993], could also regulate pectic enzyme synthesis in bacteria.

Genes regulating pectinase and other extracellular enzyme production and responding to these external stimuli are being identified in various phytopathogenic bacteria. General and specific control of pectinase and other depolymerase genes has been described in *Erwinia* spp. [for reviews see Barras *et al.*, 1994 and Chatterjee *et al.* 1991] and a complex and co-ordinated network of various genes regulating *pel* genes transcription has been defined in *E. chrysanthemi* [Reverchon *et al.*, 1991].

Factors present in plant tissues may both induce pectic enzyme genes not expressed on synthetic media [Beaulieu and van Gijsegem, 1990; Beaulieu *et al.*, 1993; Collmer *et al.*, 1991; Kelemu and Collmer, 1993; Pupillo *et al.*, 1976] and modify the ratio and kinetics of production of enzymes and isoenzymes in a manner depending on the host and also on the specific tissue [Beaulieu *et al.*, 1993; Lojkowska *et al.*, 1993; Yang *et al.*, 1992].

Studies on the chemical and structural features of plant molecules able to change the pattern of enzyme expression are at an early stage, as are the identification of bacterial regulatory genes responding to these signals. In carrot cell extract a complex oligosaccharide has been partially characterised able to induce a *pgl* locus in *Agrobacterium tumefaciens* that encodes a predicted protein with homology to known polygalacturonases [Rong *et al.*, 1994]. *pel* genes of *E. chrysanthemi* are differentially induced in the presence of plant extracts, but only in synergy with known pectate lyase inducer (KDG); partial purification and characterisation of inducing plant compounds has revealed that the active factor is thermostable, has a small molecular weight and is neither a phenolic nor a flavonoid compound [Bourson *et al.*, 1993]. In *Erwinia carotovora* subsp. *carotovora* plant signals activate the expression of *pel3* and *peh1* [Liu *et al.*, 1994] and induce genes (*aep*) that activate the production of several depolymerases [Barras *et al.*, 1994; Liu *et al.*, 1993].

The secretion apparatus is another site in which control may be exerted; an integration between the regulatory system of enzyme synthesis and the export apparatus is likely. In gram-negative bacteria, pectinase secretion occurs through a general pathway composed of two steps [for reviews see Pugseley, 1993 and Salmond, 1994]. The export apparatus has been characterised in *E.*

chrysanthemi [Lindeberg and Collmer, 1992], *E. carotovora* subsp. *carotovora* [Reeves *et al.*, 1993] and *Xanthomonas campestris* [Dow *et al.*, 1987; Dums *et al.*, 1991]; a locus which is possibly part of the export apparatus has been also described in *Pseudomonas solanacearum* [Kang *et al.*, 1994]. In *E. chrysanthemi* there is genetic evidence that regulation of the *out* genes, which are involved in pectinase and cellulase secretion, is under the control of the same gene which regulates the pectinolysis pathway [Condemine *et al.*, 1992].

Mutations of genes involved in protein export have proved that depolymerases have a role as factors of pathogenicity or virulence. Thus, mutants of *X. campestris* pv. *campestris*, *P. solanacearum*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* defective in pectinase export showed a reduced or null virulence [Beaulieu and van Gijsegem, 1992; Dow *et al.*, 1987; Kang *et al.*, 1994; Pirhonen *et al.*, 1991].

To establish the role of individual pectinases in plant diseases, mutants lacking single enzyme activity, obtained by marker exchange mutagenesis of the structural genes, have been compared to the wild type in a number of plant tests. This approach provided evidence that not all known pectinases are important pathogenicity factors. Thus, an endo-PG defective mutant of *E. carotovora* subsp. *carotovora* strain ECC71 that maintained PL and exo-PG activities, kept its virulence on tissues of different plants [Willis *et al.*, 1987]. Similarly the endo-PG of *P. solanacearum* is not required to cause tomato disease although it accelerates its development [Denny *et al.*, 1990], and the unique PL of *X. campestris* pv. *vesicatoria* has no effect on virulence [Beaulieu *et al.*, 1991; Dow *et al.*, 1989]. On the contrary the single PL produced by *P. viridiflava* determines pathogenicity [Liao *et al.*, 1988] and the PG of *A. tumefaciens* biovar 3 is necessary to cause root decay in grape seedlings [Rodriguez-Palenzuela *et al.*, 1991].

When the micro-organism produces a complex array of pectinases, each possibly comprised by multiple forms, the real contribution of a single isoenzyme to plant disease becomes more puzzling. In *E. chrysanthemi*, whose *pel* genes organisation has been thoroughly studied, five major *pel* genes and one *pem* have been characterised. Analysis of mutants deleted for all or individual genes demonstrated that only three *pel* or *pem* are important for pathogenicity on *Saintpaulia ionantha* plants [Boccara *et al.*, 1988; Boccara and Chatain, 1989]. Molecular genetic manipulations of PL production in *E. chrysanthemi* EC16 and in *pel*$^+$ *Escherichia coli* demonstrated that, while individual isoenzymes are able to produce maceration, none of the five PL isoenzymes is essential [Keen and Tamaki, 1986; Payne *et al.*, 1987; Ried and Collmer, 1988]. This analysis led to the discovery of a new set of *E. chrysanthemi* plant-inducible PLs, not produced in minimal medium containing pectate, and capable of macerating plant tissues [Beaulieu *et al.*, 1993; Collmer *et al.*, 1991; Kelemu and Collmer, 1993]. Moreover analysis of the expression of the *pel* and *pem* genes in *E. chrysanthemi* mutants suggested that the absence of one of the PL isoenzymes affects the synthesis of the others [Lojkowska *et al.*, 1993]. A study with pectinase deficient mutants of *E. chrysanthemi* showed that full expression of virulence in a given host may be due to a particular enzyme and isoenzyme combination which can differ changing the host [Beaulieu *et al.*, 1993]. Therefore for deconstructing the cell wall of some plant species or plant organs one enzyme combination may be more powerful than an other. As a consequence results from some pathogenicity tests (e.g. potato tubers) may not be generally valid. Moreover, research on *E. chrysanthemi* pointed out that the inability or ability of an individual pectinase to macerate a plant tissue is not necessarily related to a null or absolute role, respectively, in pathogenesis and that the abundance of an enzyme in plant tissue may not reflect its impact on pathogenesis [Beaulieu *et al.*, 1993].

The regulation of production of pectic enzymes by fungal pathogens was last reviewed by Collmer and Keen [1986] and Cooper [1983]. Little additional knowledge has since been obtained. At least in necrotrophic fungi the general mechanism is similar to that described in bacteria. The inducer of PG and PL, the monomer D-galacturonate, is detached from pectic polymer by basal or constitutive pectinolytic activity. Galacturonate activates transcription of inducible enzymes which, once secreted, complete the demolition of the pectic polymer. *In planta*, increasing concentrations of

nutrient sugars released from dead or dying protoplasts as well as the great availability of the inducer in the apoplastic fluid may block further production of pectic enzymes. Nutritional and environmental factors, like carbohydrate type [Leone and Van den Heuvel, 1987; Riou *et al.*, 1991], availability of phosphate and adenine nucleotide [Leone *et al.*, 1990], pH [Dean and Timberlake, 1989; Durrands and Cooper, 1988] or plant factors [Favaron and Marciano, 1992; Scott and Fielding, 1985] may interfere with the regulatory mechanism and influence the amount of production as well as the sequence of synthesis of enzyme and isoenzymatic forms. In biotrophic fungi the involvement of pectinase in pathogenesis has long been questioned. Since wall deconstruction usually causes cellular disruption, biotrophs must cause highly restricted wall degradation and this might explain the very low level of pectic enzymes produced by these parasites. Recent work reports that in the obligate biotrophic rust fungus *Uromyces viciae-fabae*, pectinase, like cellulase, is strictly controlled by the differentiation of infection structures [Frittrang *et al.*, 1992; Heiler *et al.*, 1993]. In contrast, in necrotrophs (e.g. *Botrytis allii, Rhizoctonia solani, Sclerotinia sclerotiorum*, etc.), production of pectic enzymes is not related to their differentiation and may even precede infection.

While pectic enzymes have been purified and characterised from an increasing number of fungal parasites [Dori *et al.*, 1992; Gao and Shain, 1994; Johnston and Williamson, 1992; Keon *et al.*, 1990; Pérez Artés and Tena, 1990; Prusky *et al.*, 1989; Riou *et al.*, 1992; Valsangiacomo and Gessler, 1992; Waksman *et al.*, 1991 to cite just some more recent papers], there are relatively few studies on the role of these enzymes during plant pathogenesis. Most studies have considered the ability of purified enzymes to reproduce disease symptoms or the relations between amount, timing or type of enzyme production and severity of disease symptoms [Holz and Knox-Davies, 1985; Marciano *et al.*, 1982; Marcus *et al.*, 1986; Pérez Artés and Tena, 1990; Wijesundera *et al.*, 1989]. Results are not conclusive *per se* although they indicate possible relationships and may orientate research work.

Visualisation of PG enzyme just in the tissue area in which extensive pectin breakdown or cell separation occurs has been shown in bean infected by *Colletotrichum lindemuthianum*, using a PG-binding protein as a probe, [Benhamou *et al.*, 1991] and in soybean treated with an endo-PG by *S. sclerotiorum*, using an anti-PG antibody [Favaron *et al.*, 1993b]. Convincing evidence of PG involvement in pathogenesis has been obtained with *Aspergillus flavus*. Low-virulence strains lacking a major endo-PG caused less severe disease symptoms in developing cotton bolls than one high-virulence strain that produced a major non-catabolite repressed endo-PG [Brown *et al.*, 1992; Cleveland and Cotty, 1991]. Mutagenesis gene disruption and antibodies blocking enzyme activity also have been used to study the role of pectic enzymes in pathogenesis. UV induced mutants of *Fusarium oxysporum* f. sp. *lycopersici* lacking PG and PME activity had reduced virulence on tomato [Mann, 1962] and PG-deficient mutants of *Verticillium dahliae* also produced by UV irradiation induced only delayed symptoms in cotton [Puhalla and Howell, 1975]. Chemically induced pectinase-deficient mutants of *Verticillium albo-atrum* colonised tomato plants to levels comparable to the wild type but caused reduced or delayed symptoms [Durrands and Cooper, 1988]. Antibodies against PL from *Fusarium solani* inhibited the appearance of disease symptoms on pea stems suggesting that the enzyme may be an important virulence factor; however specificity of the antiserum used was not demonstrated [Crawford and Kolattukudy, 1987]. In contrast, a strain of *Cochliobolus carbonum* in which the gene for endo-PG had been disrupted was unaffected for pathogenicity on maize [Scott-Craig *et al.*, 1990]. However this strain maintained exo-PG activity and a possible induction *in planta* of other endo-PGs was not checked.

On the basis of available data the importance of the contribution of fungal pectinase to pathogenesis must be considered case by case. Only in soft rot fungi the endo-pectinases reproducing the symptoms of the disease may be considered as factors of pathogenicity, although the contribution of other factors, e.g. other depolymerases, pH, etc., may be essential for complete disease development. Thus, the fact that *S. sclerotiorum* mutants impaired in the production of oxalic acid incited only small lesions in bean pods and leaves in spite of the high levels of pectolytic activity produced

in vitro [Godoy *et al.*, 1990] may be related to maintenance of pH levels unfavourable for enzymatic activity within the infection site. It is feasible that pathogenicity is the result of more concomitant factors, each of which may become limiting when expressed under a critical minimum. A co-ordinate increase of expression of each factor is essential for full manifestation of disease symptoms.

Plant constraints to pectin degradation

As the parasite evolved an array of enzymes to efficiently degrade the plant cell wall so the plants refined defensive tools. Among these are the plant inhibitors of pectic enzyme activity and the responses triggered by the action of pectic enzymes themselves.

The chemical nature of plant inhibitor of pectinase varies. Initially the phenolic compounds were considered the main inhibitors of pectic enzymes in microbial plant pathogenesis [Bateman and Basham, 1976]. Recently a flavan 3-ol present in high concentrations in the pericarp of unripe avocado fruits was shown to inhibit both an endo-PG and a PL produced by *Colletotrichum gloeosporioides* [Prusky *et al.*, 1989; Wattad *et al.*, 1994]. Most studies, apart from two showing the existence of protein PL and PNL inhibitors [Bock *et al.*, 1975; Bugbee, 1993], have concerned protein inhibitors of fungal PG. The occurrence of this inhibitor was reported for the first time in sweet potato tissue [Uritani and Stahaman, 1961]; later it has been discovered in many dicotyledons [for review see Hahn *et al.*, 1989] and monocotyledons [Favaron *et al.*, 1993a]. PGIPs of *Phaseolus vulgaris*, *Pyrus communis*, *Lycopersicum esculentum* and *Glycine max* have been purified and the coding genes have been cloned [Favaron *et al.*, 1994; Stotz *et al.*, 1993 and 1994; Toubart *et al.* 1992]. They are glycoproteins of 37–43 kDa, the genes possess a high degree of homology and the predicted polypeptides are rich in leucine and have a conserved position of cysteins. In spite of a strong similarity, bean and soybean PGIPs displayed different inhibitory activity when assayed against the same PGs [Favaron *et al.*, 1994]. The PGIPs are constitutively expressed in plant tissue and their distribution may vary with plant organ [Favaron *et al.*, 1994; Johnston *et al.*, 1993; Salvi *et al.*, 1990; Stotz *et al.*, 1993;]. The transcript of bean and soybean *pgip* gene is induced by wounding, elicitors and fungal infection [Bergman *et al.*, 1994; Favaron *et al.*, 1994]. Inducibility of PGIP and correlation between PGIP content of some plant organs and their resistance to fungal infection [Johnston *et al.*, 1993; Salvi *et al.*, 1990] suggest a possible involvement of this protein in plant resistance to disease. Since the level of PGIP *in planta* is low and is quickly induced by fungal infection [Bergmann *et al.*, 1994; Cervone *et al.*, 1993], it is likely that the protein plays an important role in resistance only when is overexpressed. Preliminary data show that tomato fruits of transgenic plants overexpressing the pear *pgip* gene are more resistant to *B. cinerea* [Powell *et al.*, 1994]. It has been suggested that PGIPs do not contribute directly to plant defence, but instead retard polygalacturonase-mediated hydrolysis of polypectate resulting in the formation of larger elicitor active oligogalacturonides (see below) within the infection site [Cervone *et al.*, 1989]. Further it has been hypothesised that *Phaseolus* PGIP, presenting a modular structure with leucine-rich repeat similar to that reported for proteins involved in signal transduction, may be part of the recognition system that determines host specific resistance [De Lorenzo *et al.*, 1994]. However the evidence that PGs of three races of *C. lindemuthianum* were similarly inhibited by PGIPs from four cultivars of *P. vulgaris* regardless of the race-cultivar specific interaction [De Lorenzo *et al.*, 1990] cast doubt on this hypothesis. On the other side, the observation that PG of *C. lindemuthianum* race β, likewise the fungal infection, activates β-1,3-glucanase earlier in resistant rather than susceptible isogenic lines of *P. vulgaris* [Lafitte *et al.*, 1993] indicates that a specific recognition of PG may occur. If PGIP might be involved in this process must be established in future research. If selective absorption of fungal PGs by plant tissue [De Lorenzo and Cervone, 1986] has the same molecular basis is also to be investigated.

Plants react to pathogen attack by activating defence responses. A number of molecules, called elicitors, released during the plant-pathogen interaction may incite the plant response. Among these are fragments released from the plant cell wall by

pectic enzymes action. This function of pectic enzymes was first reported by Bruce and West [1982]. Subsequently a number of endo-PGs and endo-PLs produced by bacteria [Davis *et al.*, 1984; Palva *et al.*, 1993; Yang *et al.*, 1992] and fungi [for review see De Lorenzo *et al.*, in press] have been reported to elicit defence responses in different plant species. The active fragments have been characterised as an α-1,4-linked linear oligogalacturonides and their biological activity has been associated to the DP [for review see Ryan and Farmer, 1991] and, probably, to their conformational changes induced by Ca^{2+} [Messiaen and Van Cutsem, 1993].

Defence responses induced by oligogalacturonides include synthesis of β-glucanase and chitinase, phytoalexins, proteinase inhibitors, lignin, ethylene, production of H_2O_2, etc. [for reviews see Hahn *et al.*, 1993 and Ryan and Farmer, 1991]. The induction of mRNA encoding PGIP was also observed in suspension-cultured bean cells pretreated with oligogalaturonides [Cervone *et al.*, 1993].

Beyond the ascertained function, the real involvement of these molecules in activating postinfection defences is still questioned. In fact none of the responses attributed to these molecules has shown race-cultivar specificity. Moreover the fragments are possibly also released during organogenesis by plant pectinases regulating a number of physiological processes [Branca *et al.*, 1988; Bellincampi *et al.*, 1993; Campbell and Labavitch, 1991; Cousson *et al.*, 1989; Fry *et al.*, 1993; Marfà *et al.*, 1991] and they are also likely released following interaction with symbiotic and biotrophic micro-organisms without activating detrimental plant responses. Pectic fragments therefore seem to constitute a generic non specific signal.

How the plant cell can decipher these signals as originating from incipient infection or physiological or wound repairing processes is still an open question. It may be through recognition of the different conformational structure and DP of oligogalacturonide, by perception of the relatively higher concentration of oligogalacturonides released by the pathogen in comparison to what is needed for regulating physiological processes [Messiaen and Van Cutsem, 1993] or by the need of the contemporaneous presence of other more specific signal of pathogen origin able to induce the defence responses sinergistically [Davis and Hahlbrock, 1987; Davis *et al.*, 1986; Tepper and Anderson, 1990]. Also, in the plant cell wall environment, enzymes with different properties may produce various sets of oligogalacturonides that may activate a different intensity of plant response. In soybean, a decrease of phytoalexin elicitor activity of the most active oligouronide in the presence of smaller and larger ones has been reported [Davis *et al.*, 1986] and the ability of two PGs to differentially elicit phytoalexins may refer to the different relative amounts of oligomer types released from polygalacturonate [Favaron *et al.*, 1988 and 1992].

So far most studies on oligogalacturonides have been carried out on relatively simplified systems. Products have usually been obtained after digestion of commercial substrates or purified cell wall by purified and well-characterised pectinases. During pathogenesis the pathogen usually produces a set of pectolytic enzymes, whose production and activity are in turn conditioned by composition and conditions of the plant cell wall. They act in concert or in a cascade of reactions and the contribution of plant cell wall-degrading enzymes is still unknown. Therefore amount, characteristics, stability and persistence of molecules released therein are difficult to predict as well as the type and magnitude of elicited responses. It is not excluded that as yet undiscovered or characterised active molecules may have a relevant role in some plant-pathogen interactions. For example in *Cladosporium fulvum* infecting tomato, arabinose-rich fragments derived from pectic polysaccharides by some plant or fungus endo-hydrolase are released in the apoplast [Aldington and Fry, 1992]. Since these fragments are released earlier in the incompatible interaction a possible involvement as signalling molecules that activate defence responses is conceivable.

Conclusions

The role of pectin-degrading enzymes in pathogenicity and virulence of bacterial and fungal plant parasites is, in most cases, still an open question. Also in the better studied soft-rot parasites full comprehension of the contribution of these

enzymes in plant disease is complicated by redundancy of coding genes and expression *in planta* of genes normally silent in artificial cultural conditions. Moreover, the contribution of other metabolites produced by the parasite, including different depolymerases that may act in concert with pectic enzymes [Walker *et al.*, 1994], should receive more attention in future research. The study of the complex regulatory system of bacterial pectinases and the number of functions co-ordinately driven may constitute a reliable approach to a better understanding of the pathogenic process. This approach, apart from providing speculative suggestions, should be extended to research on interactions involving fungal parasites.

The activation of plant defence responses by products of activity of pectin-degrading enzymes on plant cell wall is a well-established phenomenon. Better elucidation of their relative importance in triggering the defence responses during the infection process is expected in the near future.

The increasing knowledge and preliminary work regarding the PG-binding proteins open a promising application area in the improvement of plant resistance to some microbial plant parasites. If the hypothesis that PGIP may be part of a plant recognition system of pathogens could be proved, the potential use of this class of molecules in plant resistance improvement would be greatly extended.

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References

dington S and Fry SC (1992) Plant cell wall-lysing enzymes in the apoplast of tomato leaves infected with *Fulvia fulva*. Can. J. Bot. 70: 607–613

ras F, van Gijsegem F and Chatterjee AK (1994) Extracellular enzymes and pathogenesis of soft-rot Erwinia. Annu. Rev. Phytopathol. 32: 201–234

Bateman DF and Basham HG (1976) Degradation of plant cell walls and membranes by microbial enzymes. In: Heitefuss R and Williams PH (eds) Physiol. Plant Pathol. (pp. 316–355) Springer–Verlag, Berlin

Beaulieu C, Boccara M and van Gijsegem F (1993) Pathogenic behavior of pectinase-defective *Erwinia chrysanthemi* mutants on different plants. Mol. Plant-Microbe Interact. 6: 197–202

Beaulieu C, Minsavage GV, Canteros BI and Stall RE (1991) Biochemical and genetic analysis of a pectate lyase gene from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Plant-Microbe Interact. 4: 446–451

Beaulieu C and van Gijsegem F (1990) Identification of plant-inducible genes in *Erwinia chrysanthemi* 3937. J. Bacteriol. 172: 1569–1575

Beaulieu C and van Gijsegem F (1992) Pathogenic behaviour of several mini-Mu-induced mutants of *Erwinia chrysanthemi* on different plants. Mol. Plant-Microbe Interact. 5: 340–346

Bellincampi D, Salvi G, De Lorenzo G, Cervone F, Marfà V, Eberhard S, Darvill A and Albersheim P (1993) Oligogalacturonides inhibit the formation of roots on tobacco explants. Plant J. 4: 207–213

Benhamou N, Lafitte C, Barthe JP and Esquerré-Tugayé MT (1991) Cell surface interactions between bean leaf cells and *Colletotrichum lindemuthianum*. Cytochemical aspects of pectin breakdown and fungal endopolygalacturonase accumulation. Plant Physiol. 97: 234–244

Bergmann CW, Ito Y, Singer D, Albersheim P, Darvill AG, Benhamou N, Nuss L, Salvi G, Cervone F and De Lorenzo G (1994) Polygalacturonase-inhibiting protein accumulates in *Phaseolus vulgaris* L. in response to wounding, elicitors and fungal infection. Plant J. 5: 625–634

Boccara M and Chatain V (1989) Regulation and role in pathogenicity of *Erwinia chrysanthemi* 3937 pectin methylesterase. J. Bacteriol. 171: 4085–4087

Boccara M, Diolez A, Rouve M and Kotoujansky A (1988) The role of individual pectate lyases of *Erwinia chrysanthemi* strain 3937 in pathogenicity on saintpaulia plants. Physiol. Mol. Plant Pathol. 33: 95–104

Bock WG, Dongowski G, Göebel H und Krause M (1975) Nachweis der hemmung mikrobieller pektin- und pektatlyase durch pflanzeneigene inhibitoren. Die Nahrung 19: 411–416

Bourson C, Favey S, Reverchon S and Robert-Baudouy J (1993) Regulation of the expression of a *pelA::uidA* fusion in *Erwinia chrysanthemi* and demonstration of the synergistic action of plant extract with polygalacturonate on pectate lyase synthesis. J. Gen. Microbiol. 139: 1–9

Branca C, De Lorenzo G and Cervone F (1988) Competitive inhibition of the auxin-induced elongation by a-D-oligogalacturonides in pea stem segments. Physiol. Plant. 72: 499–504

Brown RL, Cleveland TE, Cotty PJ and Mellon JE (1992) Spread of *Aspergillus flavus* in cotton bolls, decay of intercarpellary membranes, and production of fungal pectinases. Phytopathology 82: 462–467

Bruce RJ and West CA (1982) Elicitation of casbene synthetase in castor bean. The role of pectic fragments of the

plant cell wall in elicitation by a fungal endopolygalacturonase. Plant Physiol. 69:1181–1188

Bugbee WM (1993) A pectin lyase inhibitor protein from cell walls of sugar beet. Phytopathology 83: 63–68

Bussink HJD, Brouwer KB, de Graaf LH, Kester HCM and Visser J (1991a) Identification and characterization of a second polygalacturonase gene of *Aspergillus niger*. Curr. Genet. 20: 301–307

Bussink HJD, Buxton FP and Visser J (1991b) Expression and sequence comparison of the *Aspergillus niger* and *Aspergillus tubigensis* genes encoding polygalacturonase II. Curr. Genet. 19: 467–474

Campbell AD and Labavitch JM (1991) Induction and regulation of ethylene biosynthesis by pectic oligomers in cultured pear cells. Plant Physiol. 97: 699–705

Caprari C, Richter A, Bergmann C, Lo Cicero S, Salvi G, Cervone F and De Lorenzo G (1993) Cloning and characterization of a gene encoding the *endo*polygalacturonase of *Fusarium moniliforme*. Mycol. Res. 97: 497–505

Carpita NC and Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J. 3: 1–30

Cervone F, De Lorenzo G, Caprari C, Clark AJ, Desiderio A, Devoto A, Leckie F, Nuss L, Salvi G and Toubart P (1993) The interaction between fungal *endo*polygalacturonase and plant cell wall PGIP (polygalacturonase-inhibiting protein). In: Fritig B and Legrand M (eds) Mechanisms of Plant Defence Responses (pp. 64–67) Kluwer Academic Publishers, Dordrecht, The Netherlands

Cervone F, Hahn MG, De Lorenzo G, Darvill AG and Albersheim P (1989) Host-pathogen interactions. XXXIII. A plant protein converts a fungal pathogenesis factor into an elicitor of plant defense responses. Plant Physiol. 90: 542–548

Chatterjee AK, McEvoy JL, Murata H and Collmer A (1991) Regulation of the production of pectinases and other extracellular enzymes in the soft-rotting *Erwinia* spp. In: Patil SS, Ouchi S, Mills D and Vance C (eds) Molecular Strategies of Pathogens and Host Plants (pp. 45–55) Springer-Verlag, Berlin

Cleveland TE and Cotty PJ (1991) Invasiveness of *Aspergillus flavus* isolates in wounded cotton bolls is associated with production of a specific fungal polygalacturonase. Phytopathology 81: 155–158

Collmer A, Bauer DW, He SY, Lindeberg M, Kelemu S, Rodriguez-Palenzuela P, Burr TJ and Chatterjee AK (1991) Pectic enzyme production and bacterial plant pathogenicity. In: Hennecke H and Verma DPS (eds) Advances in Molecular Genetics of Plant-Microbe Interactions. vol. I (pp. 65–72) Kluwer Academic Publishers, Amsterdam

Collmer A and Keen NT (1986) The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. 24: 383–409

Collmer A, Schoedel C, Roeder DL, Ried JL and Rissler JF (1985) Molecular cloning in *Escherichia coli* of *Erwinia chrysanthemi* genes encoding multiple forms of pectate lyase. J. Bacteriol. 161: 913–920

Condemine G, Dorel C, Hugouvieux-Cotte-Pattat N and Robert-Baudouy J (1992) Some of the *out* genes involved in the secretion of pectate lyases in *Erwinia chrysanthemi* are regulated by *kdgR*. Mol. Microbiol. 6: 3199–3212

Condemine G, Hugouvieux-Cotte-Pattat N and Robert-Baudouy J (1986) Isolation of *Erwinia chrysanthemi kduD* mutants altered in pectin degradation. J. Bacteriol. 165: 937–941

Cooper RM (1983) The mechanisms and significance of enzymic degradation of host cell walls by parasites. In: Callow JA (ed) Biochemical Plant Pathology (pp. 101–135) Wiley & Sons, New York

Cousson A, Toubart P and Tran Thanh Van K (1989) Control of morphogenetic pathway in thin cell layers of tobacco by pH. Can. J. Bot. 67: 650–654

Crawford MS and Kolattukudy PE (1987) Pectate lyase from *Fusarium solani* f.sp. *pisi*: Purification, characterization, *in vitro* translation of the mRNA, and involvement in pathogenicity. Arch. Biochem. Biophys. 258: 196–205

Davis KR, Lyon GD, Darvill AG and Albersheim P (1984) Host-pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 74: 52–60

Davis KR, Darvill AG, Albersheim P and Dell A (1986) Host-pathogen interactions. XXIX. Oligogalacturonides released from sodium polypectate by endopolygalacturonic acid lyase are elicitors of phytoalexins in soybean. Plant Physiol. 80: 568–577

Davis KR and Hahlbrock K (1987) Induction of defense responses in cultured parsley cells by plant cell wall fragments. Plant Physiol. 85: 1286–1290

Dean RA and Timberlake WE (1989) Regulation of the *Aspergillus nidulans* pectate lyase gene (*pelA*). Plant Cell 1: 275–284

De Lorenzo G, Castoria R, Bellincampi D and Cervone F Fungal invasion enzymes and their inhibition. In: Carrol G and Tudzynski P (eds) The Mycota (in press) Springer-Verlag, Berlin

De Lorenzo G and Cervone F (1986) Differential absorption rate of polygalacturonase from two races of *Colletotrichum lindemuthianum* to resistant and susceptible cultivars of *Phaseolus vulgaris* L. Annali Botanica 44: 147–154

De Lorenzo G, Cervone F, Bellincampi D, Caprari C, Clark AI, Desiderio A, Devoto A, Forrest R, Leckie F, Nuss L and Salvi G (1994) Polygalacturonase, PGIP and oligogalacturonides in cell-cell communication. Biochem. Soc. Trans. 22: 394–397

De Lorenzo G, Ito Y, D'Ovidio R, Cervone F, Albersheim P and Darvill AG (1990) Host-pathogen interactions. XXXVII. Abilities of the polygalacturonase-inhibiting proteins from four cultivars of *Phaseolus vulgaris* to inhibit the *endo*polygalacturonases from three races of *Colletotrichum lindemuthianum*. Physiol. Mol. Plant Pathol. 36: 421–435

Denny TP, Carney BF and Schell MA (1990) Inactivation of multiple virulence genes reduces the ability of *Pseudomonas solanacearum* to cause wilt symptoms. Mol. Plant-Microbe Interact. 3: 293–300

Dori S, Hershenhorn J, Solel Z and Barash I (1992

Characterization of an endopolygalacturonase associated with take-all disease of wheat. Physiol. Mol. Plant Pathol. 40: 203–210

Dow JM, Milligan DE, Jamieson L, Barber CE and Daniels MJ (1989) Molecular cloning of a polygalacturonate lyase gene from *Xanthomonas campestris* pv. *campestris* and role of the gene product in pathogenicity. Physiol. Mol. Plant Pathol. 35:113–120

Dow JM, Scofield G, Trafford K, Turner PC and Daniels MJ (1987) A gene cluster in *Xanthomonas campestris* pv. *campestris* required for pathogenicity controls the excretion of polygalacturonate lyase and other enzymes. Physiol. Mol. Plant Pathol. 31: 261–271

Dums F, Dow JM and Daniels MJ (1991) Structural characterization of protein secretion genes of the bacterial phytopathogen *Xanthomonas campestris* pathovar *campestris*: relatedness to secretion systems of other gram-negative bacteria. Mol. Gen. Genet. 229: 357–364

Durrands PK and Cooper RM (1988) The role of pectinases in vascular wilt disease as determined by defined mutants of *Verticillium albo-atrum*. Physiol. Mol. Plant Pathol. 32: 363–371

Favaron F, Alghisi P and Marciano P (1992) Characterization of two *Sclerotinia sclerotiorum* polygalacturonases with different abilities to elicit glyceollin in soybean. Plant Sci. 83: 7–13

Favaron F, Alghisi P, Marciano P and Magro P (1988) Polygalacturonase isoenzymes and oxalic acid produced by *Sclerotinia sclerotiorum* in soybean hypocotyls as elicitors of glyceollin. Physiol. Mol. Plant Pathol. 33: 385–395

Favaron F, Castiglioni C and Di Lenna P (1993a) Inhibition of some rot fungi polygalacturonases by *Allium cepa* L. and *Allium porrum* L. extracts. J. Phytopathol. 139: 201–206

Favaron F, D'Ovidio R, Porceddu E and Alghisi P (1994) Purification and molecular characterization of a soybean polygalacturonase-inhibiting protein. Planta 195: 80–87

Favaron F and Marciano P (1992) Polygalacturonase regulation in *Sclerotinia sclerotiorum*: effect of carbon source on the isoenzymatic pattern. Riv. Pat. Veg. 2: 111–123

Favaron F, Peretto R, Bonfante P and Alghisi P (1993b). Differential absorption and localization of two *Sclerotinia sclerotiorum endo*-polygalacturonases in soybean hypocotyls. Physiol. Mol. Plant Pathol. 43: 353–364

Frittrang AK, Deising H and Mendgen K (1992) Characterization and partial purification of pectinesterase, a differentiation-specific enzyme of *Uromyces viciae-fabae*. J. Gen. Microbiol. 138: 2213–2218

Fry SC, Aldington S, Hetherington PR and Aitken J (1993) Oligosaccharides as signals and substrates in the plant cell wall. Plant Physiol. 103: 1–5

Gao S and Shain L (1994) Characterization of an endopolygalacturonase produced by chestnut blight fungus. Physiol. Mol. Plant Pathol. 45: 169–179

Godoy G, Steadman JR, Dickman MB and Dam R (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris*. Physiol. Mol. Plant Pathol. 37: 179–191

Gonzales-Candelas L and Kolattukudy PE (1992) Isolation and analysis of a novel inducible pectate lyase gene from the phytopathogenic fungus *Fusarium solani* f.sp. *pisi* (*Nectria haematococca*, mating population VI). J. Bacteriol. 174: 6343–6349

Hahn MG, Bucheli P, Cervone F, Doares SH, O'Neill RA, Darvill AG and Albersheim P (1989) Roles of cell wall constituents in plant-pathogen interactions. In: Kosuge T and Nester EW (eds) Plant-Microbe Interactions. Molecular and Genetic Perspectives. Vol. 3 (pp. 131–181) McGraw-Hill, New York

Hahn MG, Cheong J J, Alba R, Enkerli J and Côté F (1993) Oligosaccharide elicitors: structures and recognition. In: Fritig B and Legrand M (eds) Mechanisms of Plant Defense Responses (pp. 99–116) Kluwer Academic Publishers, Dordrecht, The Netherlands

Heiler S, Mendgen K and Deising H (1993) Cellulolytic enzymes of the obligately biotrophic rust fungus *Uromyces viciae-fabae* are regulated differentiation-specifically. Mycol. Res. 97: 77–85

Hinton JCD, Gill DR, Lalo D, Plastow GS and Salmond GPC (1990) Sequence of the *peh* gene of *Erwinia carotovora*: homology between *Erwinia* and plant enzymes. Mol. Microbiol. 4: 1029–1036

Holz G and Knox-Davies PS (1985) Production of pectic enzymes by *Fusarium oxysporum* f. sp. *cepae* and its involvement in onion bulb rot. Phytopath. Z. 112: 69–80

Hugouvieux-Cotte-Pattat N, Dominguez H and Robert-Baudouy J (1992) Environmental conditions affect transcription of the pectinase genes of *Erwinia chrysanthemi* 3937. J. Bacteriol. 174: 7807–7818

Hugouvieux-Cotte-Pattat N and Robert-Baudouy J (1987) Hexuronate catabolism in *Erwinia chrysanthemi*. J. Bacteriol. 169: 1223–1231

Hugouvieux-Cotte-Pattat N and Robert-Baudouy J (1992) Analysis of the regulation of the *PelBC* genes in *Erwinia chrysanthemi* 3937. Mol. Microbiol. 6: 2363–2376

Itoh Y, Izaki K and Takahashi H (1980) Simultaneous synthesis of pectin lyase and carotovoricin induced by mitomycin C., nalidixic acid for ultraviolet light irradiation in *Erwinia chrysanthemi*. Agr. Biol. Chem. 44: 1135–1140

Itoh Y, Sugiura J, Izaki K and Takahashi H (1982) Enzymological and immunological properties of pectin lyases from bacteriocinogenic strains of *Erwinia carotovora*. Agr. Biol. Chem. 46: 199–205

Johnston DJ, Ramanathan V and Williamson B (1993) A protein from immature raspberry fruits which inhibits endopolygalacturonases from *Botrytis cinerea* and other micro-organisms. J. Exp. Bot. 44: 971–976

Johnston DJ and Williamson B (1992) Purification and characterization of four polygalacturonases from *Botrytis cinerea*. Mycol. Res. 96: 343–349

Kang Y, Huang J, Mao G, He L and Schell MA (1994) Dramatically reduced virulence of mutants of *Pseudomonas solanacearum* defective in export of extracellular proteins across the outer membrane. Mol. Plant-Microbe Interact. 7: 370–377

Keen NT and Tamaki S (1986) Structure of two pectate lyase genes from *Erwinia chrysanthemi* EC16 and their high

level expression in *Escherichia coli*. J. Bacteriol. 168: 595–606

Kelemu S and Collmer A (1993) *Erwinia chrysanthemi* EC16 produces a second set of plant-inducible pectate lyase isozymes. Appl. Environ. Microbiol. 59: 1756–1761

Keon JPR, Waksman G and Bailey JA (1990) A comparison of the biochemical and physiological properties of a polygalacturonase from two races of *Colletotrichum lindemuthianum*. Physiol. Mol. Plant Pathol. 37: 193–206

Lafitte C, Barthe JP, Gansel X, Dechamp-Guillaume G, Faucher C, Mazau D and Esquerré-Tugayé MT (1993) Differential induction by endopolygalacturonase of β-1,3-glucanases in *Phaseolus vulgaris* isoline susceptible and resistant to *Colletotrichum lindemuthianum* race β. Mol. Plant-Microbe Interact. 6: 628–634

Leone G, Overkamp AN, Kreyenbroek MN, Smit E and Van den Heuvel J (1990) Regulation by orthophosphate and adenine nucleotides of the biosynthesis of two polygalacturonases by *Botrytis cinerea in vitro*. Mycol. Res. 94: 1031–1038

Leone G and Van den Heuvel J (1987) Regulation by carbohydrates of the sequential *in vitro* production of pectic enzymes by *Botrytis cinerea*. Can. J. Bot. 65: 2133–2141

Liao C-H (1991) Cloning of pectate lyase gene *pel* from *Pseudomonas fluorescens* and detection of sequence homologous to *pel* in *Pseudomonas viridiflava* and *Pseudomonas putida*. J. Bacteriol. 173: 4386–4393

Liao C-H, Hung H-Y and Chatterjee AK (1988) An extracellular pectate lyase is the pathogenicity factor of the soft-rotting bacterium *Pseudomonas viridiflava*. Mol. Plant-Microbe Interact. 1: 199–206

Liao C-H, McCallus DE and Wells JM (1993) Calcium-dependent pectate lyase production in the soft-rotting bacterium *Pseudomonas fluorescens*. Phytopathology 83: 813–818

Liao C-H, Sasaki K, Nagahashi G and Hicks KB (1992) Cloning and characterization of a pectate lyase gene from the soft-rotting bacterium *Pseudomonas viridiflava*. Mol. Plant-Microbe Interact. 5: 30–308

Lindeberg M and Collmer A (1992) Analysis of eight *out* genes in a cluster required for pectic enzyme secretion by *Erwinia chrysanthemi*: Sequence comparison with secretion genes from other gram-negative bacteria. J. Bacteriol. 174: 7385–7397

Liu Y, Chatterjee A and Chatterjee AK (1994) Nucleotide sequence and expression of a novel pectate lyase gene *(pel-3)* and a closely linked endopolygalacturonase gene *(peh-1)* of *Erwinia carotovora* subsp. *carotovora* 71. Appl. Environ. Microbiol. 60: 2545–2552

Liu Y, Murata H, Chatterjee A and Chatterjee AK (1993) Characterization of a novel regulatory gene *aepA* that controls extracellular enzyme production in the phytopathogenic bacterium *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. 6: 299–308

Lojkowska E, Dorel C, Reignault P, Hugouvieux-Cotte-Pattat N and Robert-Baudouy J (1993) Use of GUS fusion to study the expression of *Erwinia chrysanthemi* pectinase genes during infection of potato tubers. Mol. Plant-Microbe Interact. 6: 488–494

Mann B (1962) Role of pectic enzymes in the *Fusarium* wilt syndrome of tomato. Trans. Br. Mycol. Soc. 45: 169–178

Marciano P, Di Lenna P and Magro P (1982) Polygalacturonase isoenzymes produced by *Sclerotinia sclerotiorum in vivo* and *in vitro*. Physiol. Plant Pathol. 20: 201–212

Marcus L, Barash I, Sneh B, Koltin Y and Finkler A (1986) Purification and characterization of pectolytic enzymes produced by virulent and hypovirulent isolates of *Rhizoctonia solani* Kuhn. Physiol. Mol. Plant Pathol. 29: 325–336

Marfà V, Gollin DJ, Eberhard S, Mohnen D, Darvill AG and Albersheim P (1991) Oligogalacturonides are able to induce flowers to form on tobacco explants. Plant Journal 1: 217–225

Messiaen J and Van Cutsem P (1993) Defense gene transcription in carrot cells treated with oligogalacturonides. Plant Cell Physiol. 34: 1117–1123

Nasser HW, Condemine G, Plantier R, Anker D and Robert-Baudouy J (1991) Inducing properties of analogs of 2-keto-3-deoxygluconate on the expression of pectinase genes of *Erwinia chrysanthemi*. FEMS Microbiol. Lett. 81: 73–78

Nikaidou N, Kamio Y and Izaki K (1993) Molecular cloning and nucleotide sequence of the pectate lyase gene from *Pseudomonas marginalis* N6301. Biosci. Biotech. Biochem. 57: 957–960

Palva TK, Holmstrom KO, Heino P, Palva ET (1993) Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. 6: 190–196

Payne JH, Schoedel C, Keen NT and Collmer A (1987) Multiplication and virulence in plant tissues of *Escherichia coli* clones producing pectate lyase isozymes PLb and PLe at high levels and of an *Erwinia chrysanthemi* mutant deficient in PLe. Appl. Environ. Microbiol. 53: 2315–2320

Pérez Artés E and Tena M (1990) Purification and characterization of pectic enzymes from two races of *Fusarium oxysporum* f. sp. *ciceri* differing in virulence to chickpea (*Cicer arietinum* L.). Physiol. Mol. Plant Pathol. 37: 107–124

Perombelon MCM and Kelman A (1980) Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. 18: 361–387

Pirhonen M, Saarilahti H, Karlsson M-B and Palva ET (1991) Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. Mol. Plant-Microbe Interact. 3: 276–283

Powell ALT, D'hallewin G, Hall BD, Stotz H, Labavitch JM and Bennett AB (1994) Glycoprotein inhibitors of fungal polygalacturonases: expression of pear PGIP improves resistance in transgenic tomatoes (abstr.). Plant Physiol. 105: 880

Prusky D, Gold S and Keen NT (1989) Purification and characterization of an endopolygalacturonase produced by *Colletotrichum gloeosporioides*. Physiol. Mol. Plant Pathol. 35: 121–133

Pugsley AP (1993) The complete general secretory pathway in Gram-negative bacteria. Microbiol. Rev. 57: 50–108

Puhalla JE and Howell CR (1975) Significance of endopolygalacturonase activity to symptoms expression of verticillium wilt cotton, assessed by the use of mutants

of *Verticillium dahliae* Kleb. Physiol. Plant Pathol. 7: 147–152

Pupillo P, Mazzucchi U and Pierini G (1976) Pectic lyase isozymes produced by *Erwinia chrysanthemi* Burkh. *et al.* in polypectate broth or in Dieffenbachia leaves. Physiol. Plant Pathol. 9: 113–120

Reeves PJ, Whitcombe D, Wharam S, Gibson M, Allison G, Bunce N, Barallon R, Douglas P, Mulholland V, Stevens S, Walker D and Salmond GPC (1993) Molecular cloning and characterization of 13 *out* genes from *Erwinia carotovora* subspecies *carotovora*: genes encoding members of a general secretion pathway (GSP) widespread in Gram-negative bacteria. Mol. Microbiol. 8: 443–456

Reverchon S, Nasser W and Robert-Baudouy J (1991). Characterization of *kdgR*, a gene of *Erwinia chrysanthemi* that regulates pectin degradation. Mol. Microbiol. 5: 2203–2216

Ried JL and Collmer A (1988) Construction and characterization of an *Erwinia chrysanthemi* mutant with directed deletions in all of the pectate lyase structural genes. Mol. Plant-Microbe Interact. 1: 32–38

Riou C, Freyssinet G and Fevre M (1991) Production of cell wall-degrading enzymes by phytopathogenic fungus *Sclerotinia sclerotiorum*. Appl. Environ. Microbiol. 57: 1478–1484

Riou C, Freyssinet G and Fevre M (1992) Purification and characterization of extracellular pectinolytic enzymes produced by *Sclerotinia sclerotiorum*. Appl. Environ. Microbiol. 58: 578–583

Rodriguez-Palenzuela P, Burr TJ and Collmer A (1991) Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3. J. Bacteriol. 173: 6547–6552

Rong L, Carpita NC, Mort A and Gelvin SB (1994) Soluble cell wall compounds from carrot roots induce the *picA* and *pgl* loci of *Agrobacterium tumefaciens*. Mol. Plant-Microbe Interact. 7: 6–14

Ryan CA and Farmer EE (1991) Oligosaccharide signals in plants: a current assessment. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 651–674

Salmond GPC (1994) Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. 32: 181–200

Salvi G, Giarrizzo F, De Lorenzo G and Cervone F (1990) A polygalacturonase-inhibiting protein in the flowers of *Phaseolus vulgaris* L. J. Plant Physiol. 136: 513–518

Sauvage C, Franza T and Expert D (1990) Iron as a modulator of pathogenicity of *Erwinia chrysanthemi* on *Saintpaulia ionantha*. In: Hennecke H and Verma DPS (eds) Advances in Molecular Genetics of Plant-Microbe Interactions. Vol. I (pp. 94–97) Kluwer Academic Publishers, Amsterdam

Schell MA, Roberts DP and Denny TP (1988) Analysis of the *Pseudomonas solanacearum* polygalacturonase encoded by *pglA* and its involvement in phytopathogenicity. J. Bacteriol. 170: 4501–4508

Scott SW and Fielding AH (1985) Differences in pectolytic enzyme patterns induced in *Sclerotinia trifoliorum* by different legume host species. Trans. Br. Mycol. Soc. 84: 317–324

Scott-Craig JS, Panaccione DG, Cervone F and Walton JD (1990) Endopolygalacturonase is not required for pathogenicity of *Cochliobolus carbonum* on maize. Plant Cell 2: 1191–1200

Stotz HU, Contos JJA, Powell ALT, Bennet AB and Labavitch JM (1994) Structure and expression of an inhibitor of fungal polygalacturonases from tomato. Plant Mol. Biol. 25: 607–617

Stotz HU, Powell ALT, Damon SE, Greve CG, Bennet AB and Labavitch JM (1993) Molecular characterization of a polygalacturonase inhibitor from *Pyrus communis* L. cv. Bartlett. Plant Physiol. 102: 133–138

Tepper CS and Anderson AJ (1990) Interactions between pectic fragments and extracellular components from the fungal pathogen *Colletotrichum lindemuthianum*. Physiol. Mol. Plant Pathol. 36: 147–158

Tomizawa H and Takahashi H (1971) Stimulation of pectolytic enzyme formation of *Erwinia aroideae* by nalidixic acid, mitomycin C and bleomycin. Agr. Biol. Chem. 35: 191–200

Toubart P, Desiderio A, Salvi G, Cervone F, Daroda L, De Lorenzo G, Bergmann C, Darvill AG and Albersheim P (1992) Cloning and characterization of the gene encoding the endopolygalacturonase-inhibiting protein (PGIP) of *Phaseolus vulgaris* L. Plant J. 2: 367–373

Tsuyumu S and Chatterjee AK (1984) Pectin lyase production in *Erwinia chrysanthemi* and other soft-rot *Erwinia* species. Physiol. Plant Pathol. 24: 291–302

Uritani I and Stahmann MA (1961) Pectolytic enzymes of *Ceratocystis fimbriata*. Phytopathology 51: 277–285

Valsangiacomo C and Gessler C (1992) Purification and characterization of an exo-polygalacturonase produced by *Venturia inaequalis*, the causal agent of apple scab. Physiol. Mol. Plant Pathol. 40: 63–77

Waksman G, Keon JPR and Turner G (1991) Purification and characterization of two endopolygalacturonases from *Sclerotinia sclerotiorum*. Biochim. Biophys. Acta 1073: 43–48

Walker DS, Reeves PJ and Salmond GPC (1994) The major secreted cellulase, CelV, of *Erwinia carotovora* subsp. *carotovora* is an important soft rot virulence factor. Mol. Plant-Microbe Interact. 7: 425–431

Wattad C, Dinoor A and Prusky D (1994) Purification of pectate lyase produced by *Colletotrichum gloeosporioides* and its inhibition by epicatechin: a possible factor involved in the resistance of unripe avocado fruits to anthracnose. Mol. Plant-Microbe Interact. 7: 293–297

Wijesundera RLC, Bailey JA, Byrde RJW and Fielding AH (1989) Cell wall degrading enzymes of *Colletotrichum lindemuthianum*: their role in the development of bean anthracnose. Physiol. Mol. Plant Pathol. 34: 403–413

Willis JW, Engwall JK and Chatterjee AK (1987) Cloning of genes for *Erwinia carotovora* subsp. *carotovora* pectolytic enzymes and further characterization of the polygalacturonases. Phytopathology 77: 1199–1205

Yang Z, Cramer CL and Lacy GH (1992) *Erwinia carotovora* subsp. *carotovora* pectic enzymes: *in planta* gene activation and roles in soft-rot pathogenesis. Mol. Plant-Microbe Interact. 5: 104–112