Use of GUS Fusion to Study the Expression of *Erwinia chrysanthemi* Pectinase Genes During Infection of Potato Tubers

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***Erwinia chrysanthemi* mutants, containing transcriptional fusion of one of the major pectinase genes (*pem, pelA, pelB, pelC, pelD, pelE*) with the reporter gene encoding β-glucuronidase activity, were studied for their ability to synthesize pectinases and to cause disease symptoms after inoculation of potato tubers and of *in vitro* plants. There were no differences in growth of all the tested mutants, and all caused soft rot. The *pelB::uidA, pelC::uidA,* and *pelE::uidA* mutants exhibited lower pectate lyase activity than the wild type. Pectinase genes could be separated into two groups according to their expression level, i.e., 1) the moderately expressed genes: *pelA, pelB, pelC,* and *pem;* 2) the highly expressed genes: *pelD* and *pelE*. Expression of some *pel::uidA* fusions were different in inoculated tubers and in bacterial culture. The *pelA::uidA* fusion showed a 23-fold higher GUS activity in tubers compared to synthetic medium. Conversely, the *pelE* and *pem* expression is favored after inoculation of *in vitro* plants. The comparison of the pectinase activity and of the expression of the fusions in bacterial culture and *in planta* suggests that one or more plant factor(s) modulates the expression of these genes.**

Additional keywords: pectate lyase, pectin methyl esterase, soft rot.

The enterobacterium *Erwinia chrysanthemi* causes soft rot disease in a wide range of plants. *E. chrysanthemi* produces several extracellular enzymes that can degrade plant cell walls and, among these, pectinolytic enzymes seem to play a key role in the pathogenicity of *Erwinia* (Basham and Bateman 1975; Collmer and Keen 1986; Perombelon and Kelman 1980). Pectin is at first demethylated by the pectin methyl esterase (PME, *pem* gene), then pectate lyases (PL, *pel* genes) cuts the internal glycosidic bonds of polygalacturonic acid by a β-elimination reaction. *E. chrysanthemi* strain 3937 produces one PME and five isoenzymes of PL (Bertheau *et al.* 1984). The nomenclature of PL isoenzymes refers to their pI: from the most acidic, PLa, to the most basic, PLe. Cloning and analysis of the six genes *pem, pelA, pelB, pelC, pelD,* and *pelE* revealed that the PME and all the PL isoenzymes are encoded by an independent transcriptional unit (Reverchon *et al.* 1986; Plastow 1988; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1992). These genes are arranged into two clusters, widely separated on the bacterial chromosome (Hugouvieux-Cotte-Pattat *et al.* 1992); the genes *pelB* and *pelC* form one cluster, *pelA, pelD, pelE,* and *pem* belong to the second cluster.

Production of pectinases is inducible in the presence of either pectin, its demethylated derivative polygalacturonic acid (PGA) or the monomer, galacturonate. Analysis of mutants blocked at different stages of pectinolysis has shown that the real intracellular inducers are catabolic products, mainly 2-keto-3-deoxygluconate (Condemine *et al.* 1986; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1987; Nasser *et al.* 1991). Physiological studies demonstrated that PL synthesis is subject to other types of regulation, such as catabolite repression or induction during the late exponential growth phase (Hugouvieux-Cotte-Pattat *et al.* 1986, 1992). PL synthesis is also regulated by temperature, oxygen tension (Perombelon and Kelman 1980; Hugouvieux-Cotte-Pattat *et al.* 1992), and iron concentration (Sauvage *et al.* 1991). Therefore, it appears that the regulation of the PL synthesis is complex, both at the physiological and the genetic level, and probably requires several regulatory systems. Operon fusions between the six pectinase genes and the reporter gene *uidA* (coding for the β-glucuronidase, GUS) were constructed *in vitro* and introduced into the bacterial genome (Hugouvieux-Cotte-Pattat *et al.* 1992). Using these gene fusions, we investigated the expression of the *pel* and *pem* genes of the *E. chrysanthemi* following inoculation of potato tubers.

RESULTS

Pathogenicity of mutants on potato tubers and on plantlets.

Potato tubers were inoculated with *E. chrysanthemi* wild-type (WT) strain and with the different *pel::uidA* or

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pem::uidA mutants. Inoculation with all these mutants caused typical soft macerated tissue around the inoculation site. No significant difference of virulence, estimated by the extent of maceration, was observed during the 3 days following inoculation (Fig. 1). Rotting progressed quickly and linearly at the rate of about 1 mm every 4 hr. Therefore, under these experimental conditions, the inactivation of one pectinase gene does not significantly affect the rotting ability of the bacteria. Likewise, *in vitro* plants inoculated with different mutants showed disease symptoms similar to those plants inoculated with the wild-type strain (Table 1).

Growth of the mutants with a *pel::uidA* or *pem::uidA* fusion in tubers and in plant tissue.

During infection by the WT strain of *Erwinia*, we found that bacteria stay mainly in the rotted tissue. Few (<5%) were found immediately around the macerated tissue (data not shown). Therefore, in all subsequent experiments, the rotted part plus 2 mm of the adjacent tissue were taken and referred to as rotten tissue. Bacterial multiplication was followed by an estimation of the bacterial cell concentration in the rotted tissue; that is the increase of the total *Erwinia* population at each inoculation site (Fig. 2). Analysis of the multiplication of the mutants in tubers showed that their growth rate is as rapid as that of the wild type (Fig. 2). Similar results were obtained when bacteria were isolated from the *in vitro* plants (Table 1). No significant difference between these strains could be detected by the analysis of variance test. All mutants were actively dividing and appeared in the stationary phase of growth on the second day following inoculation in tubers (Fig. 2).

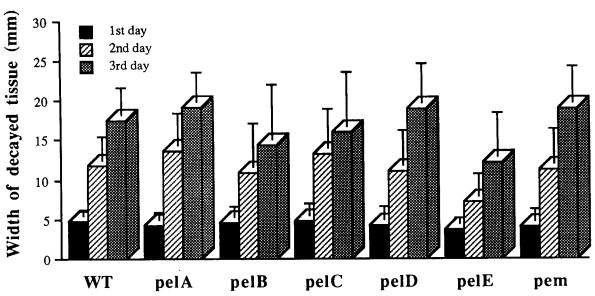

Fig. 1. Comparison of the pathogenicity of *Erwinia chrysanthemi* 3937 wild-type and *pel* or *pem* mutants on potato tubers. Potato tubers were inoculated using sterile pipet tips containing 50 µl of bacterial suspension (2×10^9 cfu/ml) and incubated in a dew chamber at 30° C. Width of decayed tissue was determined during the 3 days following inoculation. Error bars indicate standard deviation calculated from three independent experiments.

Table 1. Pathogenicity of *Erwinia chrysanthemi* wild-type and *pel* or *pem* mutants in *in vitro* plants[a]

Strain	Disease index	No. bacteria 10^9 cfu/ml
3937 (WT)	4.3	0.8
A1888 (*pelA::uidA*)	3.8	0.5
A1787 (*pelB::uidA*)	ND[b]	ND
A1880 (*pelC::uidA*)	3.8	0.5
A1798 (*pelD::uidA*)	3.7	0.7
A1881 (*pelE::uidA*)	4.0	0.3
A1789 (*pem::uidA*)	4.1	0.7

[a] *In vitro* plants were inoculated by 50 µl of bacterial suspension (10^8 cfu/ml) and incubated in a phytotron at 25° C, with relative humidity of 90%. Disease index and number of bacteria were determined 3 days after inoculation. The crushed surface of the plant was covered with 50 µl of suspension of *E. chrysanthemi* (10^8 cfu/ml). The disease index was calculated as a mean value for 12 seedlings inoculated with the same strain 3 days after inoculation.

[b] Not determined.

Pectinase synthesis during infection.

To compare the ability of the mutants to synthesize pectinases in tuber tissue, total PL activity at each inoculation site was calculated. The values were obtained by multiplication of the PL activity per gram of tissue by the weight of rotted tissue. The wild-type strain and all the mutants showed an increase in total PL activity at the inoculation site during the 3 days following inoculation. Pectinolytic activity began to increase significantly from the second day after inoculation (Fig. 3). Comparison between the mutants and the wild-type strain shows that WT, *pelA*,

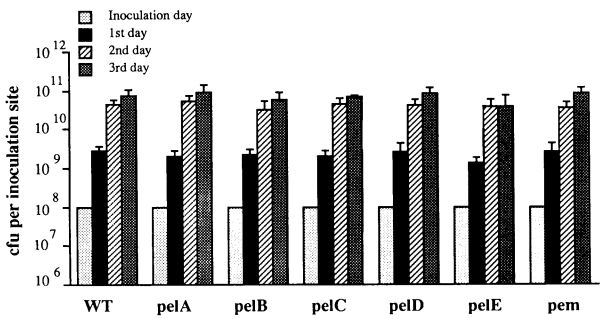

Fig. 2. Kinetics of bacterial multiplication on potato tubers. Potato tubers were inoculated with wild-type strain and the six mutants containing a fusion between one pectinase gene and the reporter gene *uidA*, as described in Figure 1. Bacteria were isolated from the rotted tissue and dilution plating was performed each day. The number of colony-forming units per gram fresh weight was multiplied by the rotted tissue weight to obtain the total number of colony-forming units in the inoculation site. Error bars indicate standard deviation calculated from three independent experiments.

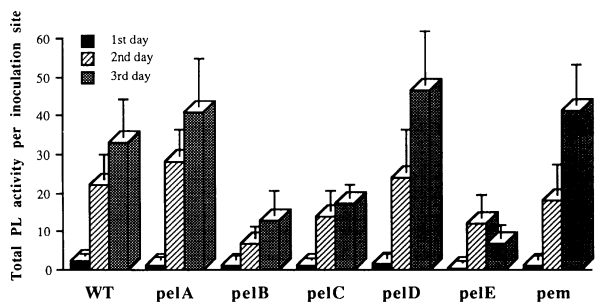

Fig. 3. Synthesis of total pectinase after inoculation of potato tubers. The wild-type strain and the six mutants containing a fusion between pectinase genes and the reporter gene *uidA* were inoculated into potato tubers as described in Figure 1. PL activity was determined each day. Histograms show the total PL activity detected at the inoculation site. These values were obtained by multiplication of the PL activity per gram of tissue by the weight of rotted tissue. Error bars indicate standard deviation calculated from two independent experiments.

pelD, and *pem* mutants synthesize and secrete similar levels of pectinolytic enzymes (Fig. 3). However, *pelB, pelC,* and *pelE* mutants synthesize reduced levels of PL. Analysis of variance shows that *pelB, pelC,* and *pelE* mutants produce significantly lower levels of PL per inoculation site during the 3 days after inoculation (Fig. 3).

Comparison of the PL specific activity, expressed as μmol of unsaturated products liberated per minute per milligram of bacterial dry weight, in inoculated potato tubers showed that all mutants synthesize less PL than the wild type (Fig. 4). However, a significantly lower level of synthesis is observed only in the *pelB, pelC,* and *pelE* mutants (Fig. 4). Despite this reduced PL activity, similar levels of maceration were obtained with all the strains (Fig. 1).

From the expression of the *uidA* fusions during the infection process it is possible to differentiate two groups: moderately expressed fusions in: *pelA, pelB, pelC,* and *pem,* up to 500 units (Fig. 5A) and highly expressed fusions in *pelD* and *pelE,* up to 4,000 units (Fig. 5B). However, we observed no relationship between the level of expression of each *pel* gene, as estimated by the fusion expression (Fig. 5), and the remaining PL activity in the mutants (Fig. 4).

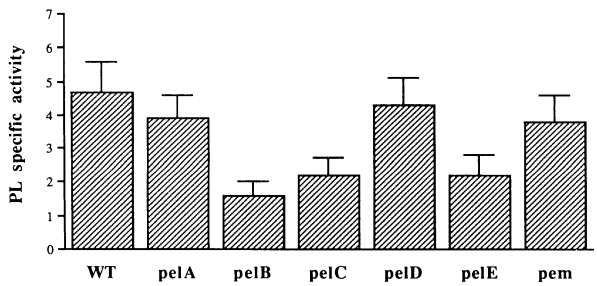

Fig. 4. Pectinase-specific activity after inoculation of potato tubers. The wild-type strain and the six mutants containing a fusion between pectinase genes and the reporter gene *uidA* were inoculated into potato tubers as described in Figure 1. PL activity was determined 48 hr after inoculation. Specific activity of PL is expressed as μmol of unsaturated products liberated per minute per milligram of bacterial dry weight. Error bars indicate standard deviation calculated from two independent experiments.

Expression of *pel* and *pem* genes in bacterial cultures and in tubers.

The results shown in Tables 2 and 3 were obtained from the experiments designed specifically to compare the expression of the *pel* genes in bacterial culture with those in infected tubers or plants. Tubers, *in vitro* plants, and synthetic medium with or without tissue extract were inoculated with the same bacterial culture. The GUS specific activity of mutants with transcriptional fusion in *pel* or *pem* gene ranged from 13 to 346 units, depending on the gene, during growth in M63 medium + glycerol + PGA (Table 2). When the mutants were grown in the same medium complemented with tuber tissue extract, the expression level of the *pelA, pelB,* and *pelE* fusions was not affected. The expression of *pelC* and *pem* fusions was slightly increased (threefold), but the most striking effect, a 16-fold increase, was observed for *pelD.*

We also investigated the expression of *pel* and *pem* genes in tubers. Strain *pelA* showed a 23-fold increase of GUS activity in tubers compared with the activity in M63 me-

Table 2. Expression of the fusions *pel::uidA* and *pem::uidA* in bacterial cultures and in potato tubers[a]

	Specific activity			Induction	
	in cultures	in tubers		in cultures	in tubers
Fusion strain	**A**	**B**	**C**	**B/A**	**C/B**
A1888 (*pelA::uidA*)	13	13	306	1	23
A1787 (*pelB::uidA*)	138	120	147	1	1
A1880 (*pelC::uidA*)	200	639	355	3	<1
A1798 (*pelD::uidA*)	120	1,887	2,929	16	2
A1881 (*pelE::uidA*)	346	466	1,412	1	3
A1789 (*pem::uidA*)	160	520	342	3	1

[a] The results were obtained from one experiment designed specifically to compare the expression of the *pel* genes in bacterial culture and in infected tubers. Tubers and synthetic medium with or without tuber tissue extract were inoculated with the same bacterial culture. This experiment was repeated and gave similar results to those shown in the Table. Specific activity of GUS is expressed as nmol of *p*-nitrophenol liberated from *p*-nitrophenyl-β-D-glucouronide per minute per milligram of bacterial dry weight. A - M63 minimal medium + glycerol + PGA; B - M63 minimal medium + glycerol + PGA + tubers extract; C - expression of the fusion 2 days after inoculation of potato tubers.

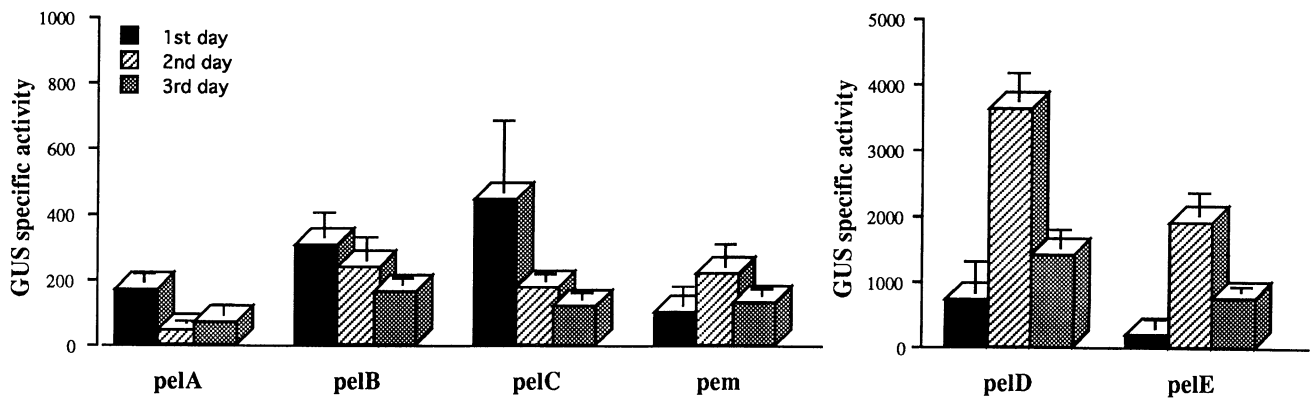

Fig. 5. Expression of *pel::uidA* and *pem::uidA* gene fusions after inoculation of potato tubers. The wild-type strain and the six different mutants containg a fusion between pectinase genes and the reporter gene *uidA* were inoculated into potato tubers as described in Figure 1. The GUS activity was determined each day. Specific activity of GUS is expressed as nmol of *p*-nitrophenol liberated per minute per milligram of bacterial dry weight. Error bars indicate standard deviation calculated from two independent experiments.

dium containing potato tuber extract. The other mutants showed only slight differences in expression of *pel* and *pem* fusions in inoculated tubers compared with their expression in M63 medium containing tuber extracts (Table 2).

To check if the stimulation of the *pelA* gene is tissue specific, we investigated the *pel* expression on *in vitro* plantlet of potato (Table 3). We found that *pelA* fusion is expressed at about the same level in bacterial cultures and in *in vitro* plants. This suggests that there is a compound(s), able to turn on specifically the *pelA* expression, contained in the tubers but not in the plantlet tissue. Conversely, we found that after inoculation of plants, the *pelE* and *pem* expressions were about 20-fold higher than the expression of these genes in bacterial cultures or in infected tubers (Tables 2 and 3). This indicates that one, or more, specific activator(s) of *pelE* and *pem* genes is present in the plantlet.

DISCUSSION

Mutants of *E. chrysanthemi*, with transcriptional fusion of *pel* and *pem* genes with the reporter gene *uidA*, were used to investigate the importance of the individual pectinases in pathogenicity on potato. During the first 3 days after inoculation of the potato tubers we determined the progression of: 1) disease severity, 2) bacterial growth, 3) pectate lyase activity due to the nonmutated genes, and 4) GUS activity, which indicates the expression level of the fused pectinase gene.

All the tested mutants were unaffected in their ability to macerate potato tuber or *in vitro* plantlets. Potato tissue was described earlier as very sensitive to maceration by a wide range of *E. chrysanthemi* mutants (Beaulieu and Van Gijsegem 1992). Even a nonpathogenic bacteria such as *E. coli* containing an overexpressed *E. chrysanthemi pelE* gene is able to macerate potato tuber (Payne *et al.* 1987). However, the ability of *Erwinia* to cause disease symptoms on tubers depends on the environmental conditions in which the test is performed (Perombelon and Kelman 1980; Maher and Kelman 1983). Tests of the pathogenicity are usually performed in conditions favorable for the tissue maceration (30° C, high humidity, limited oxygen availability). The resistance mechanisms in tuber tissue are inhibited by anaerobic conditions (Vayda and Schaeffer 1988; Lyon 1989; Łojkowska and Hołubowska 1992; Vayda *et al.* 1992).

Comparison of the total PL activity in the rotted tissue of potato tuber indicates that the mutants *pelB, pelC,* and *pelE* synthesize less enzyme than the wild type. However, the fact that this decrease in PL activity did not affect the disease severity (Fig. 1) suggests that PL are synthesized in excess by *E. chrysanthemi* for tuber maceration. Therefore the pectinolytic capacity of *E. chrysanthemi* seems "overevaluated" for maceration of potato tubers, and the suppression of one pectinase has no visible effects on disease severity. Similar results were obtained for *in vitro* plants of potato. However, when inoculation was performed with lower levels of inoculum (10^6 cfu/ml) *pelE* and *pem* mutants showed lower levels of disease severity (data not shown). It should be noted that when tested on different plants different results are obtained. For instance *pelA, pelD, pelE,* and *pem* mutants showed a decrease in virulence on the *in vitro* plantlets of *Saintpaulia ionantha* (Boccara *et al.* 1988; Boccara and Chatain 1989). Moreover, different isoenzymes may be important, depending on the host plant (Beaulieu and Van Gijsegem 1992).

All of the screened mutants inoculated in potato tubers showed growth rates similar to that of the wild type and reached densities above 10^{10} cfu per site of inoculation after 2 days. The growth rate of the five *pel::uidA* or of *pem::uidA* mutants was also unchanged when tested on *in vitro* plants or on synthetic medium (Hugouvieux-Cotte-Pattat *et al.* 1992).

Wild-type and all tested mutants show an increase in pectinolytic activity during tubers infection (Fig. 3). This probably results from an additive effect of bacterial multiplication and induction of PL synthesis after inoculation. As shown for *in vitro* cultures, PL synthesis increased during the late exponential growth phase of the bacteria (Hugouvieux-Cotte-Pattat *et al.* 1992). The fact that *pelA, pelB,* and *pelC* genes showed maximum expression on the first day, while for *pelD, pelE,* and *pem* this occurred on the second day (Fig. 5A,B) suggests that these genes may be expressed sequentially, for example, depending on the major type of substrate available in the tuber tissue.

At first, the PL-specific activity in each mutant was thought to indicate the contribution of the corresponding enzyme to total PL activity (Fig. 5). As in the case of cultures on synthetic medium (Hugouvieux-Cotte-Pattat *et al.* 1992), we observed a decrease in PL activity in all the *pel* mutants tested on potato tubers. But, the reduction in PL activity due to each PL isoenzyme is not additive, since the sum of the decreases in activity, due to the absence of a single PL isoenzymes each time, is higher than 100%. These results suggest that the absence of one of the PL isoenzymes affects the synthesis of the others.

When bacteria are grown on synthetic medium, the *pelA* fusion expression is barely detectable and only reaches a level that is about 10-fold lower than the expression

Table 3. Expression of the fusions *pel::uidA* and *pem::uidA* in bacterial cultures and in plants[a]

	Specific activity in cultures		in plants	Induction factor in cultures	in plants
Strain	**A**	**B**	**C**	**B/A**	**C/B**
A1888 (*pelA::uidA*)	13	13	22	1	2
A1787 (*pelB::uidA*)	138	200	ND[b]	1	ND
A1880 (*pelC::uidA*)	200	555	515	3	1
A1798 (*pelD::uidA*)	120	1,012	1,665	3	2
A1881 (*pelE::uidA*)	346	924	22,533	3	24
A1789 (*pem::uidA*)	160	537	10,789	3	20

[a] The results were obtained from one experiment designed specifically to compare the expression of the *pel* genes in bacterial culture and in infected *in vitro* plants. *In vitro* plants and synthetic medium with or without plant tissue extract were inoculated with the same bacterial culture. This experiment was repeated and gave similar results to those shown in the Table. Specific activity of GUS is expressed as nmol of *p*-nitrophenol liberated from *p*-nitrophenyl-β-D-glucuronide per minute per milligram of bacterial dry weight. A - M63 minimal medium + glycerol + PGA; B - M63 minimal medium + glycerol + PGA + plant extract; C - expression of the fusion 2 days after inoculation of *in vitro* plants.

of *pelB, pelC,* and *pem* (Table 2). The acidic pectate lyase PLa represents a small contribution to the total PL activity *in vitro* and is unable to elicit potato cell death and maceration when incubated alone on potato disks (Barras *et al.* 1987). In contrast, after infection of potato tubers, *pelA* shows a similar level of expression as *pelB, pelC,* or *pem.* Our results indicate that the expression of *pelA* is 23-fold higher when bacteria invade potato tubers than when bacteria are grown in synthetic medium, even in inducing conditions (Table 2). The addition of potato tuber extract to the bacterial culture is unable to stimulate *pelA* expression. Moreover, inoculation of the potato *in vitro* plants did not stimulate the *pelA* expression (Table 3). These results lead us to suppose that stimulation of the *pelA* expression is tissue specific. Some plant factor(s) present in tuber tissue could activate the *pelA* gene. Alternatively, the chemical composition of tuber tissue or specific metabolites may contribute to the assignment of a particular induction. However, the fact that potato tuber extract alone is unable to stimulate *pelA* activity indicates that either the potential inducing factor is unstable, or that PLa synthesis is controlled by another environmental condition(s) present in tubers, such as oxygen concentration. Bourson *et al.* (1993) demonstrated that *pel* genes are induced in the presence of carrot extract, but only in synergy with the known pectate lyase inducer KDG (2-keto-3-deoxygluconate). However, in our experiment the expression of *pelA* is poorly induced by carrot extract (unpublished data).

The *pelB* and *pelC* fusions reached the same level of expression when grown in synthetic media (Table 2). In contrast, *pelC* is expressed to a greater extent than *pelB* both in synthetic medium with tuber extract or within the tuber (Table 2). The *pelB* and *pelC* genes are located at the same locus and present a high degree of homology, suggesting a recent duplication of the genes (Tamaki *et al.* 1988; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1992). Preston *et al.* (1992) found that PLb and PLc isoenzymes catalyze identical depolymerization of pectin. Therefore, the PLc isoenzyme alone could be sufficient for the degradation of potato tuber tissue, and it may be used preferentially to PLb in tubers tissue. Other results show that *pelB* or *pelC* mutants caused similar disease symptoms in *Saintpaulia*, as the wild-type strain (Boccara *et al.* 1988). This might suggest that PLb and PLc could replace each other in a process of polygalacturonic acid degradation or that these isoenzymes are not necessary for the maceration of certain plants.

The observation that *pel* genes show different expressions in bacterial cultures, in tubers and in potato plantlets could explain, in part, the intriguing fact that *E. chrysanthemi* secretes at least five pectate lyases in order to macerate the plant tissue.

MATERIALS AND METHODS

Bacterial strains and growth condition.

The *E. chrysanthemi* strains used in this study were the wild-type strain 3937 and a set of the derivatives mutants with transcriptional fusions of the six pectinase genes, with the reporter gene encoding GUS activity: A1888 (*pelA::uidA*), A1787 (*pelB::uidA*), A1880 (*pelC::uidA*), A1798 (*pelD::uidA*), A1881 (*pelE::uidA*), and A1789 (*pem::uidA*) (Hugouvieux-Cotte-Pattat *et al.* 1992). Bacteria were grown in M63 synthetic medium with glycerol as a carbon source. Liquid cultures were grown in a shaking incubator for 24 hr at 30° C, and were then washed with M63 and adjusted to 2×10^9 colony-forming units/ml (cfu/ml).

Plant material.

Potato tubers (*Solanum tuberosum* L. 'Mona Lisa'), were purchased locally.

Pathogenicity tests.

Tubers were washed and immersed twice for 20 min in 5% sodium hypochlorite, rinsed in sterile deionized water, and air-dried under a laminar flow hood. Sterile polypropylene pipet tips containing 50 ml of bacterial suspension (2×10^9 cfu/ml) were inserted, in a randomized manner, into the tuber parenchyma to the depth of 10 mm (Maher and Kelman 1983; Austin *et al.* 1988). Usually four pipet tips were used per tuber: Three contained suspensions of different mutants and the fourth contained the wild-type strain. Five tubers were inoculated with each mutant and incubated at 30° C in a dew chamber at 100% relative humidity. Disease severity was determined every day for 3 days following inoculation. Tubers were sliced vertically through each inoculation point. The width of decayed tissue was measured and taken as the characteristic of disease severity.

"*In vitro*" plants were grown for 4 wk at a temperature of 25° C, with a relative humidity of 90% and 18 hr of light per day. The third leaf and petiole of each plant, which have about 10 leaves, were crushed with forceps, and the crushed surface was covered with 50 μl of suspension of *E. chrysanthemi* (10^8 cfu/ml). Disease severity was evaluated by the following disease rating system: no disease symptoms = 0; single leaf wilting = 1; several leaves wilting = 2; wilting of entire plant = 3; wilting and stem rot, but seedling survives inoculation = 4; stem rot and death of seedling = 5. The disease index was calculated 3 days after inoculation as a mean value for 12 seedlings inoculated with the same strain. Estimation of the multiplication of bacteria and of the GUS activity were performed 3 days after inoculation as described below.

Bacterial multiplication in tuber and *in vitro* plant tissue.

For determination of bacterial multiplication, samples of rotted tissue from five tubers or 12 plants (stems and leaves) inoculated with the same strain were collected and 1 g of tissue was homogenized in 10 ml of M63. Colony-forming units were determined by plating appropriate dilutions on agar plates with or without kanamycin (20 μg/ml).

Enzyme assays.

Assays of pectate lyase and β-glucuronidase were performed on toluenized extracts of infected tissues (tissue from five tubers or 12 plants inoculated with the same strain was collected). Pectate lyase activity was determined by the degradation of PGA to unsaturated products that

absorb at 235 nm (Moran *et al.* 1968). Specific activity of PL is expressed as μmol of unsaturated products liberated per minute per milligram of bacterial dry weight. β-Glucuronidase activity was measured by the degradation of *p*-nitrophenyl-β-D-glucuronide into *p*-nitrophenol that absorbs at 405 nm (Bardonnet and Blanco 1992). Specific activity of GUS is expressed as nmol of products liberated per minute per milligram of bacterial dry weight. Figures 3 and 4 show the total PL or GUS activity detected in rotted tissue. These values were obtained by multiplication of the PL activity per gram of tissue by the weight of the rotted tissue.

Tuber and plant extracts.

Crude tissue extracts were obtained by crushing tubers or *in vitro* plants tissue (stems and leaves). Homogeneous tissues were clarified by centrifugation and subsequent filtration on membranes of decreasing porosity. The extract was sterilized by the final filtration on a 0.22-μm porosity membrane. The extracts were stored in aliquots at −20° C for several months.

Statistical analysis.

The results are the average of three independent experiments. The maceration abilities, growth rates, and the expression of the PL and GUS activities of the mutants and wild-type strain were statistically compared using a 2 factor (time and strain) analysis of variance test.

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