MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 5, No. 4, pp. 340-346, 1992

Pathogenic Behavior of Several Mini-Mu-Induced Mutants of *Erwinia chrysanthemi* on Different Plants

Carole Beaulieu and Frédérique Van Gijsegem

Département de Biologie Moléculaire, Université Libre de Bruxelles, B1640 Rhode St-Genèse, Belgium.
Received 6 August 1991. Revised 3 April 1992. Accepted 10 April 1992.

Insertion mutagenesis with Mu dIIPR3 as mutator agent and subsequent screening of the mutants on *Pisum sativum* identified virulence factors in *Erwinia chrysanthemi* strain 3937. Seven mutants showing reduced virulence on pea were isolated. These mutants were characterized by three different phenotypes: deficiency in secretion of pectate lyases and endoglucanases; reduction of growth in minimal media; and lack of a 40-kDa outer membrane protein. Using four plant species, we assessed the virulence of these mutants and of previously derived Mu dIIPR3 mutants with insertions in plant-inducible genes. The mutant with reduced growth in minimal media showed reduced virulence only on one host plant. Mutants defective in the export of pectate lyases and endoglucanases showed reduced virulence on all host plants tested. Most mutants with alterations in plant-inducible genes and the mutant with a 40-kDa outer membrane protein showed reduced virulence on all host plants tested except potato tuber. Other mutants in plant-inducible genes were reduced in virulence on one or two host plants, were as virulent as the wild type, or were supervirulent on specific hosts.

Genetic studies of the interactions between plants and pathogenic bacteria have been done with a wide variety of microorganisms (Daniels *et al.* 1988). Three main strategies have been used. The first entails the isolation of mutants defective in the production of factors known or suspected to be important in pathogenicity and the subsequent characterization of the pathogenic behavior of these mutants. In the second strategy, genes involved in pathogenicity are identified among randomly mutagenized bacteria screened on plants for a defect in virulence. The third approach involves the identificaton of plant-inducible genes and the assessment of their involvement in pathogenicity (Osbourn *et al.* 1987; Beaulieu and Van Gijsegem 1990).

The first and third strategies have been used to elucidate pathogenicity mechanisms in *Erwinia chrysanthemi* Burkholder, McFadden and Dimock, a plant pathogenic enterobacterium that causes soft rot on harvested crops and on growing plants. *E. chrysanthemi* strain 3937, which was isolated from *Saintpaulia ionantha* H. Wendl. (Lemattre and Narcy 1972) has been studied extensively. Several pathogenicity factors such as the production and the secretion of a set of depolymerizing enzymes (Kotoujansky 1987), some components of the bacterial envelope such as lipopolysaccharides (Schoonejans *et al.* 1987) and proteins induced in iron-starved conditions (Expert and Toussaint 1985), and a functional iron uptake system (Enard *et al.* 1988) were identified in this strain.

In the genome of strain 3937, the fusion transposon, mini-Mu dIIPR3, was used to identify several genes that are inducible by *S. ionantha* plant extract (Beaulieu and Van Gijsegem 1990). Most of these plant-inducible genes are needed for the full expression of virulence. Pathogenicity factors encoded by noninducible genes were not detected. Random mutagenesis followed by a direct screening on the plant would be a powerful method for searching for putative pathogenicity factors. However, to use this strategy, one must work with a plant-bacteria interaction model in which the host plant is easily cultivated and propagated axenically and which reproduces clear disease symptoms. After inoculation of *E. chrysanthemi* strain 3937 on different plants, we found that pea (*Pisum sativum* L.) fulfilled these conditions. Random mutagenesis with Mu dIIPR3 and the subsequent screening of bacteria containing mini-Mu on pea plantlets identified mutants showing reduced virulence on pea. In this paper, we present a characterization of these mutants. To determine the role of *E. chrysanthemi* virulence factors in various hosts, we also compared the ability of these mutants and previously derived Mu dIIPR3 induced mutants, with insertions in plant-inducible genes, to infect or macerate different host plants.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used in this study are listed in Table 1.

Media. Bacterial strains were kept on L agar (Maniatis *et al.* 1982). Ceria broth (Glansdorff 1965) was the mineral base of all minimal media. Carbon sources were added to minimal media at a concentration of 0.2%. Antibiotics were used at a concentration of 20 μg/ml. We solidified media by adding Difco agar (15 g/L).

Mini-Mu insertion mutagenesis and isolation of mutants whose virulence is affected on pea. The mini-Mu insertion mutagenesis of strain 3937 was done as previously described (Beaulieu and Van Gijsegem 1990). A lysate of a Mu *c*ts (Mu dIIPR3), dilysogenic *Escherichia coli* (Migula)

Address correspondence to C. Beaulieu: Département de Biologie, Université de Sherbrooke, Sherbrooke, Québec, Canada, J1K 2R1.
Present address of F. Van Gijsegem: Laboratoire de Biologie Moléculaire des relations plantes-micro-organismes, INRA-CNRS, 31326 Castanet-Tolosan, Cédex France.

© 1992 The American Phytopathological Society

Castellani and Chalmers strain was obtained by thermoinduction. This lysate was used to infect an overnight culture of strain 3937. After 15 min of adsorption, L broth was added, and the mixture was incubated for 2 hr at 30° C to allow expression of the antibiotic resistance gene. Mini-Mu lysogens were selected at 35° C on L plates supplemented with chloramphenicol. Lysogens were pooled, diluted, and added to *S. ionantha* plant extract; they were incubated for 6 hr at 30° C. Kanamycin was then added, and the incubation was continued overnight. We diluted the overnight culture to obtain isolated colonies from L plates supplemented with chloramphenicol. These colonies were purified on chloramphenicol plates, and their virulence was tested on pea plantlets as described below.

Plant culture conditions, plant extract preparation, and pathogenicity tests. Plants of *S. ionantha* 'Blue Rhapsody' were cultured axenically as described by Enard *et al.* (1988). *S. ionantha* plant extract was prepared as previously described (Beaulieu and Van Gijsegem 1990). Axenic pea plantlets were obtained by soaking seeds of *P. sativum* 'Proval' in a solution of sodium hypochloride (0.5 M) and Triton X-100 (0.5%) for 30 min and then soaking three times in sterile tap water for 5 min. The sterilized seeds were germinated on 0.6% agarose plates, then transferred in tubes containing a Murashige and Skoog (MS) salt solution (Flow Laboratories, McLean, VA) solidified by the addition of 0.8% agarose.

Plant tissue maceration tests were performed on potato tubers and on etiolated witloof chicory leaves. We made holes in plant organs by using a pipetman tip. The wounded tissues were inoculated with 50 μl (potato tuber) or 5 μl (witloof chicory leaf) of a bacterial suspension (1×10^8 cfu/ml). We scored potato tuber maceration by measuring the weight of rotten tissues after 120 hr of maceration at 30° C, whereas we estimated witloof chicory maceration by measuring the maximal length of macerated tissues after 24 hr of incubation at 30° C. The maceration abilities of the mutants and the wild-type strain were statistically compared by an analysis of variance test (Little and Hills 1978).

Pathogenicity tests were performed on axenic *S. ionantha* and *P. sativum*. Two-month-old *S. ionantha* and 8-cm-high *P. sativum* were infected as described by Enard *et al.* (1988). At least 26 plantlets were inoculated with each of the different mutants. After an infection period of 15 (*S. ionantha*) and 8 (*P. sativum*) days, the number of systemically infected plants was scored. Statistical comparisons between the virulence rates of the mutants and the wild-type strain were done by a chi-square test (Little and Hills 1978).

Characterization of the mutants. Endoglucanase, pectate lyase, and protease activities were detected with the cup-plate technique (Beaulieu and Van Gijsegem 1990). To determine the localization of the pectate lyase isoenzymes, we grew bacteria in the presence of polygalacturonate as the sole carbon source, and we analyzed culture supernatants as well as contents of the periplasmic fraction (Andro *et al.* 1984) for pectinolytic activity (Van Gijsegem 1986). Pectate lyase isoenzymes were electrofocused in ultra-thin polyacrylamide gels (pH gradient from 3 to 10) according to the procedure of Bertheau *et al.* (1984).

Protein and lipopolysaccharide composition of the outer membrane was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The ability to grow on iron-starved medium was tested on an LB plate containing ethylenediamine-*N*,*N*′-bis(2-hydroxyphenylacetic acid) (EDDA). We estimated the number of mini-Mu insertions in the genome of the mutants by hybridizing the *Hpa*I-digested genomic DNA of the mutants to ^{32}P-labeled λ *c*I540::Mu dIIPR3 DNA (Desmet *et al.* 1989). All these methods were previously described (Beaulieu and Van Gijsegem 1990). Transductions with the *E. chrysanthemi* generalized transducing phage Phi EC-2 were performed as described by Resibois *et al.* (1984).

Growth curves. To compare growth of the mutants and the wild-type strain on different carbon sources (glucose, galacturonate, and polygalacturonate), we prepared an inoculum by incubating bacteria in LB for 18 hr. The cells were collected by centrifugation at $5,000 \times g$ for 10 min and resuspended in $MgSO_4$ (10 mM). The suspension was diluted 100 times in liquid minimal media containing different sugars as the sole carbon source. We monitored the growth rate at 30° C by measuring at intervals the turbidity

Table 1. Bacterial strains used in this study

Strain	Mutant class	Phenotype or relevant characteristics	Reference
RH7001	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7002	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7003	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7004	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7005	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7006	3	Pin^-	Beaulieu and Van Gijsegem 1990
RH7007	4	Pin^-	Beaulieu and Van Gijsegem 1990
RH7008	2	Pin^-	Beaulieu and Van Gijsegem 1990
RH7009	3	Pin^-	Beaulieu and Van Gijsegem 1990
RH7010	4	Pin^-	Beaulieu and Van Gijsegem 1990
RH7011	2	Omp^-	This study
RH7012	3	Rgr^-	This study
RH7013	ND[z]	Out^-	This study
RH7014	ND	Out^-, contains two mini-Mu insertions	This study
RH7015	1	Out^-	This study
RH7016	ND	Contains two mini-Mu insertions	This study
RH7017	ND	Contains two mini-Mu insertions	This study
3937	NA[z]	Wild-type strain	Lemattre and Narcy 1972

[z] ND = not determined; NA = not applicable.

of the cultures at 620 nm; a CP1 colorimeter (Science Essentials, Germany) was used.

RESULTS

Isolation of mutants whose virulence is affected on pea. *E. chrysanthemi* strain 3937 can efficiently infect fast-growing pea plantlets; therefore, we used this host to search for mutants showing reduced virulence after transposon mutagenesis. We used a derivative of Mu bacteriophage, Mu dIIPR3 (Ratet and Richaud 1986), to randomly mutagenize strain 3937. The transposase gene was deleted from this mini-Mu, therefore, the transposon is stable on insertion. The transposon carries a constitutive chloramphenicol resistance gene and a promotorless kanamycin resistance gene. On insertion, gene fusion can occur between the *nptI* gene and promotors of *E. chrysanthemi*. Plant-inducible genes have been previously found in *E. chrysanthemi* (Beaulieu and Van Gijsegem 1990) by the identification of mutants that express resistance to kanamycin only in the presence of plant extract.

By inoculating bacteria onto pea plantlets, we determined the virulence of 2,500 chloramphenicol-resistant clones that survived incubation in plant extract in the presence of kanamycin. The clones that caused no or localized symptoms after a 15-day incubation in three independent experiments were further analyzed (22 strains). Seven of the 22 strains caused systemic disease at a level significantly lower than that of the wild-type strain ($P < 0.05$).

Characterization of reduced virulence mutants on pea. Depolymerizing enzymes play an essential role in pathogenicity by degrading plant cell wall constituents and causing plant tissue maceration. Using the cup-plate technique, we tested the seven mutants for their ability to produce and secrete pectinases, cellulases, and proteases. All mutants produced and secreted proteases (data not shown). However, three of them (RH7013, RH7014, and RH7015) showed no or very small lysis zones around the spot of inoculation when they were grown on plates containing polygalacturonate or carboxymethyl cellulose, the respective substrates of pectate lyases and endoglucanases. Electrofocusing of culture supernatants or crude cell extracts from the mutants revealed that the seven mutants produced all five pectate lyase isoenzymes. However, pectate lyase activity in strains RH7013, RH7014, and RH7015 was associated with periplasmic fractions (Fig. 1). These mutants appeared to be affected in the export of pectinases (Out$^-$ mutants).

Because depolymerizing enzyme activities and the secretion of these enzymes did not seem altered in mutants RH7011, RH7012, RH7016, and RH7017, other factors known to be important in the plant-bacteria interaction were examined: the outer membrane composition (Schoonejans *et al.* 1987), the iron assimilation system (Enard *et al.* 1988), the growth rate (Hinton *et al.* 1989), the catabolism of galacturonate and polygalacturonate (Beaulieu and Van Gijsegem 1990), and the bacterial motility (Pirhonen *et al.* 1991).

Analysis of the outer membrane revealed no apparent alteration in the lipopolysaccharide composition (data not shown). However, in strain RH7011, the major band of the outer membrane protein pattern was absent (Fig. 2). The molecular weight of this protein was about 40 kDa. RH7011 was called Omp$^-$. The growth of the four mutants (RH7011, RH7012, RH7016, and RH7017) on iron-deficient medium was comparable to the growth of the wild-type strain; this suggests that the iron assimilation system in the mutants was not affected (data not shown). The growth rate of these mutants in LB and in minimal media supplemented with glucose, galacturonate, or polygalacturonate as the sole carbon source was also determined. Despite its alteration in membrane composition, the growth of strain RH7011 was comparable to the growth of RH7016 and RH7017, and the growth of these mutants was equivalent to the wild-type strain in all minimal media tested. The growth of RH7012 and the wild-type strain was comparable in LB; however, RH7012 showed a delayed lag phase in all minimun media tested, and the maximal optical density (OD) of RH7012 cultures after an overnight incubation was always less than that of the wild-type cultures (data not shown). RH7012 was called Rgr$^-$ for reduced growth. The motility of all mutants was confirmed by microscopic observation.

The number of mini-Mu insertions inside the bacterial genome of the mutants was estimated by hybridizing DNA of λ *c*I540::Mu dIIPR3 (Desmet *et al.* 1989), a λ derivative carrying Mu dIIPR3, to the *Hpa*I-restricted total DNA of the mutants. Because Mu dIIPR3 contained no *Hpa*I restriction site and no homology was detected between the

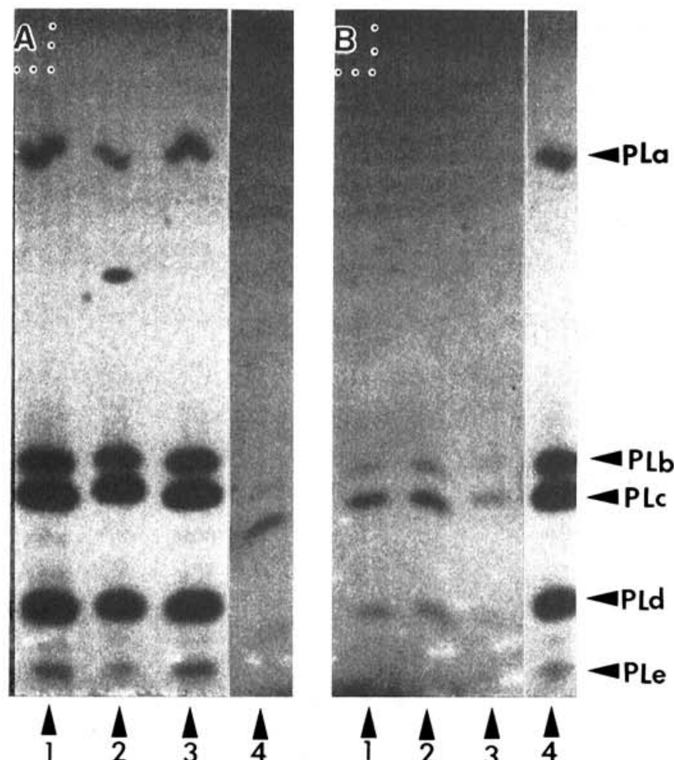

Fig. 1. Electrofocusing on a thin polyacrylamide gel of **A**, periplasmic fractions and **B**, supernatants from *Erwinia chrysanthemi* cultures grown in a minimal medium containing polygalacturonate as the sole carbon source. 1, RH7013; 2, RH7014; 3, RH7015; and 4, 3937. PLa–PLe, pectate lyases a–e.

total DNA of the wild-type strain 3937 and the λ DNA, the number of hybridized bands reflected the number of mini-Mu insertions. The DNA of the λ derivative hybridized to a single band of total DNA from RH7011, RH7012, RH7013, and RH7015. Two or more bands in the genomic DNA of the Out$^-$ mutant RH7014 and of the mutants RH7016 and RH7017 hybridized with the labeled λ *c*I540::Mu dIIPR3 DNA, indicating that more than one transposon event occurred (data not shown). The hybridization pattern was different within the mutants. Mutants harboring more than one mini-Mu insertion were not further characterized. All mutants harboring a single Mu insertion were resistant to kanamycin on rich and minimal media; thus, the *nptI* gene was expressed even in the absence of plant extract.

We analyzed linkage between the Mu DIIPR3 insertion and the Out$^-$, Rgr$^-$, and Omp$^-$ phenotypes by transducing the chloramphenicol-resistant phenotype in the wild-type strain 3937 with the transducing phage Phi EC-2. Two transductants for each mutation were characterized. *Hpa*I-digested DNA of transductants and of parental mutants was hybridized with pACYC177 (Chang and Cohen 1978), a plasmid carrying the same kanamycin resistance gene as the one from Mu DIIPR3. This plasmid hybridized bands of the identical molecular weight in transductants and parental mutants (data not shown). Transductants and parental mutants displayed the same characteristics of growth rate, secretion of pectinases, electrophoresis pattern of outer membrane proteins, and pathogenicity on witloof chicory leaf. Linkage study was also achieved with some plant-inducible (Pin$^-$) mutants (RH7001, RH7005, and RH7006). Again, similar hybridization patterns and phenotypes in transductants and parental mutants were observed.

Behavior of the mutants on different plant systems. Using the mini-Mu transposon Mu dIIPR3, we isolated two sets of mutants. The first set included the mutants described above with reduced virulence on pea. The second set included the mutants that harbored an insertion in plant-inducible genes; most of these Pin$^-$ mutants showed a decreased virulence on *S. ionantha* (Beaulieu and Van Gijsegem 1990). To determine if these bacterial factors of virulence, which are required for the full expression of virulence on one host, are also as important for virulence in other host plants, we analyzed, on different plants, the virulence of the following mutants: the Out$^-$ mutant RH7015, the Rgr$^-$ mutant RH7012, the Omp$^-$ mutant RH7011, and 10 mutants in plant-inducible genes (RH7001–RH7010). Virulence of the mutants was tested on two *in vitro* propagated plantlets (*P. sativum* and *S. ionantha*) and on two isolated plant organs (potato tuber and witloof chicory leaf). In Figure 3, virulences of the mutants and the wild-type strain on these four plants are compared.

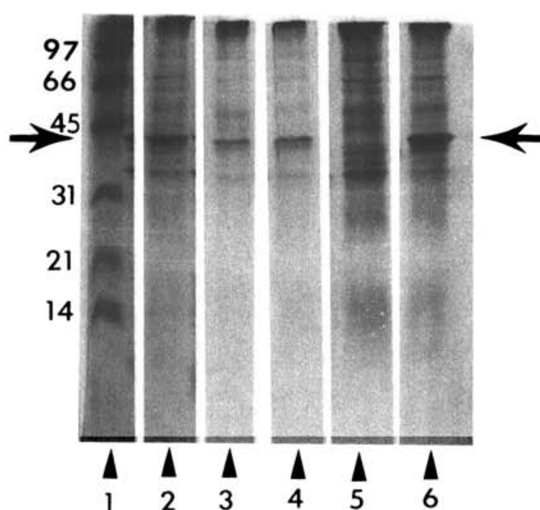

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of outer membrane proteins from *Erwinia chrysanthemi* strains. 1, Molecular weight standards; 2, RH7012; 3, RH7016; 4, RH7015; 5, RH7011; 6, 3937. Numbers at the left indicate the molecular mass standards in kilodaltons. The arrow indicates the position of the major band of the outer membrane proteins; the band is absent in strain RH7011.

Transposon-induced mutants were grouped into four classes according to their pathogenic behavior on plants (Table 1). The first class includes mutants showing reduced virulence on all plants tested. Only one mutant belongs to this class, RH7015, which is defective in the export of pectate lyases and endoglucanases.

The second class includes mutants showing reduced virulence on *S. ionantha*, *P. sativum*, and witloof chicory leaf but which do not significantly differ from the wild-type strain in their ability to macerate potato tuber. Most of the mutants tested (7 out 13) could be classified in this group. The Omp$^-$ mutant (RH7011) and six Pin$^-$ mutants (RH7001, RH7002, RH7003, RH7004, RH7005, RH7008) fit into this class.

The third class includes mutants showing reduced virulence on only one or two hosts. Three mutants belong to this class. Pin$^-$ mutants RH7006 and RH7009 behaved like the wild-type strain 3937 on pea plantlets and potato tubers but induced less severe symptoms on *S. ionantha* and on chicory leaf. The Rgr$^-$ mutant RH7012 efficiently macerated the two plant organs tested but lacked the capability to spread inside the two intact plants tested.

The fourth class includes bacterial strains as virulent as the wild-type strain 3937 on all plants and strains that are supervirulent on specific hosts. Two Pin$^-$ mutants are in this last class: strain RH7007, which showed no significant difference to the wild type in its ability to provoke soft rot on all plants tested, and strain RH7010, which was supervirulent on chicory leaf and on pea plantlets.

DISCUSSION

In this paper, we show how a random mutagenesis method can be used to identify putative pathogenicity genes in *E. chrysanthemi* strain 3937. By using a Mu derivative (Mu dIIPR3 carrying a promotorless kanamycin fusion marker) as a mutator agent, we isolated mutants with reduced virulence on pea plantlets at a frequency of 0.3%. This frequency is similar to the recovery frequency of mutants impaired in virulence obtained in other mutagenesis studies with different species of plant pathogenic bacteria (Boucher *et al.* 1985; Hinton *et al.* 1989). However, in these other mutagenesis studies, several auxotrophic mutants were found; on the other hand, auxotrophic mutants were

lost during incubation in the plant extracts used in the present study.

Mutants reduced in virulence on pea were characterized by three different phenotypes. Three mutants are impaired in the secretion of pectate lyases and endoglucanases; another lacks a major outer membrane protein; and the fifth one shows reduced growth in minimal media.

As the secretion of depolymerizing enzymes, the bacterial cell envelope seems to play a crucial role in this plant-bacteria interaction. Lipopolysaccharide molecules (Schoonejans *et al.* 1987) and proteins induced in iron-starved conditions (Expert and Toussaint 1985) have previously been shown to be essential to pathogenicity. In this study, we showed a correlation between the disappearance of a major outer membrane protein and a reduction in virulence. The role of these components in the recognition of the pathogen by the plant warrants further investigations.

The third type of mutant isolated is impaired in growth in minimal media. This phenotype is not dependent on the carbon source used. In a similar transposon mutagenesis experiment done with a related species, *Erwinia carotovora* subsp. *atroseptica*, a mutant showing both a reduced growth rate and a reduced virulence, was also isolated (Hinton *et al.* 1989). However, some differences between the two mutants are evident. In contrast to the *E. c.* subsp. *atroseptica* mutant, the *E. chrysanthemi* mutant is unaffected during its growth in LB medium and retains its capacity to efficiently macerate potato tubers.

Besides auxotrophic mutants and the mutant described above, another class of mutants of *E. c.* subsp. *atroseptica* that showed a decrease in both the synthesis and the secretion of pectic enzymes (Pep^-) was isolated (Hinton *et al.* 1989). Such Pep^- mutants involved in a global regulation of pectinolysis were not found in *E. chrysanthemi.* However, nothing is known about the regulation of the *pep* gene(s), and it is possible that, like the *pel* genes (Beaulieu and Van Gijsegem 1990), those *pep* genes are not induced in the presence of plant extract.

The host range of most *E. chrysanthemi* strains is usually not limited to the plant species from which the bacterium was isolated (Dickey 1979, 1981; Janse and Ruissen 1988). That is the case for strain 3937, which causes soft rot disease on several plant species. *E. chrysanthemi* can also cause soft rot on harvested crops, and when artificially inoculated onto isolated plant organs, strain 3937 can efficiently macerate plant tissues. Potato tubers were very sensitive to maceration by *E. chrysanthemi.* All mutants tested

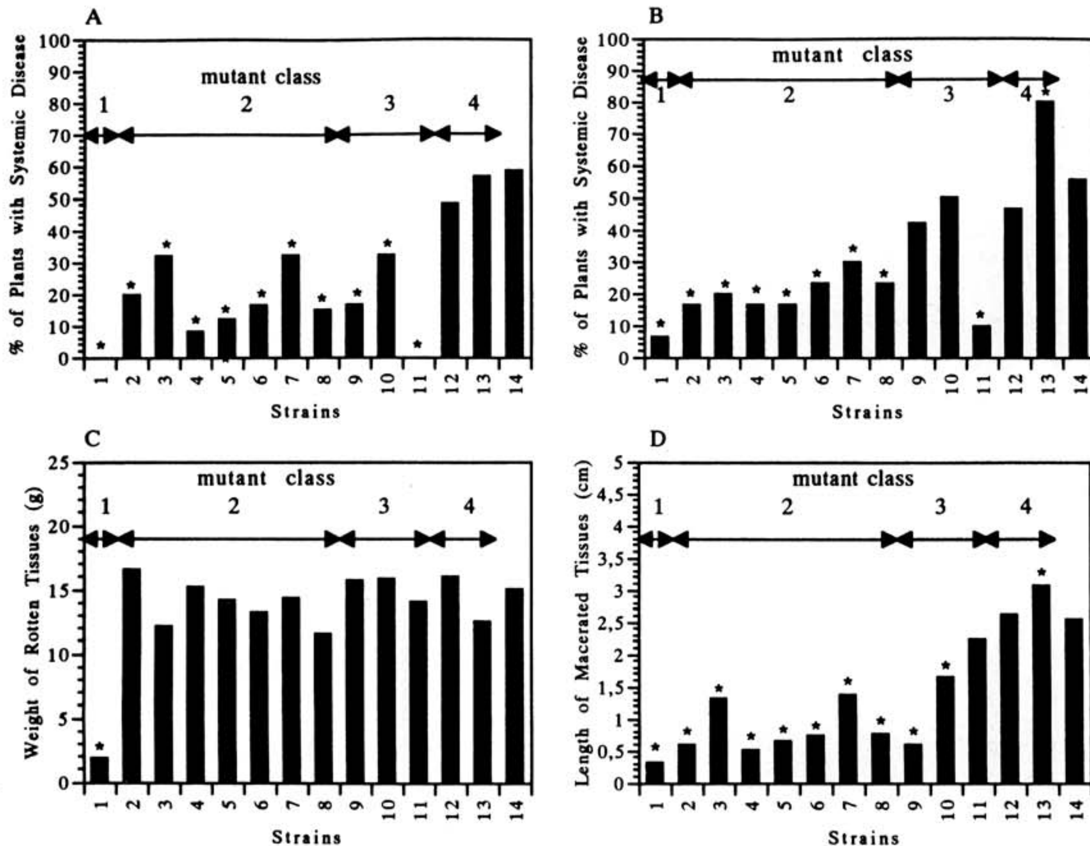

Fig. 3. Pathogenic behavior of *Erwinia chrysanthemi* mutants of **A,** *Saintpaulia ionantha* plantlet; **B,** *Pisum sativum* plantlet; **C,** potato tuber; **D,** witloof chicory leaf. In A and B, virulence was evaluated by comparing the ability of the mutants and the wild-type strain to cause systemic disease on plantlets. Each strain was inoculated on at least 26 different plantlets in three independent experiments. In C, virulence was estimated by measuring the weight of rotten potato tissues. The values on the histogram are the means of 18 repetitions of three independent experiments. In D, bacterial virulence was compared by measuring the length of macerated tissues. The values on the histogram are the means of 30 repetitions of three independent experiments. The stars indicate values statistically different from the wild-type value ($P < 0.05$). Strain 1, RH7015; 2, RH7001; 3, RH7002; 4, RH7003; 5, RH7004; 6, RH7005; 7, RH7008; 8, RH7011; 9, RH7006; 10, RH7009; 11, RH7012; 12, RH7007; 13, RH7010; and 14, 3937.

except the Out⁻ mutant were unaffected in their ability to macerate the tubers. Payne *et al.* (1987) even showed that a nonpathogenic bacteria such as *E. coli* containing an overexpressed *E. chrysanthemi pelE* gene could macerate potato tuber; this suggests that the production of pectinases is sufficient to rot potatoes. Potato tubers, therefore, do not appear to be a very informative plant model for studying the role of putative virulence genes in *E. chrysanthemi*. In contrast, witloof chicory leaf, another isolated organ, was a good system for discriminating mutants.

The gene products of most of the plant-inducible genes and the mutated gene of the Omp⁻ mutant are needed for a full expression of virulence in all plant systems tested except potato tuber; this indicates that the pathogenic factors involved in these cases are implicated in general mechanisms needed for plant infection.

Only one mutant, RH7012, retained its ability to efficiently macerate the two plant organs but was unable to spread inside the two growing plants tested. However, because this mutant displayed a reduced growth rate in minimal media, it is not known if the gene involved in the mutation is necessary for the spread of the bacteria inside the plant or if the bacterial efficiency in infecting potato tuber and chicory leaf simply reflects the encounter by the bacteria of more appropriate nutritive conditions in those plants.

Differential behavior on plants was not only observed in mutants altered in growth or catabolic functions. RH7006, a mutant in a plant-inducible gene, is fully pathogenic on pea plantlets and potato tubers but exhibits reduced virulence on the two other plants tested. Thus, some bacterial genes are necessary for pathogenicity on some hosts, whereas they are dispensable on others. The chemical composition of plants or some specific plant metabolites could contribute to the assignment of a particular host range to an *E. chrysanthemi* strain.

Two Pin⁻ mutants that were previously shown to be as virulent as the parental strain on *S. ionantha* were also tested on different plants. One of them behaved like the wild-type strain, whereas strain RH7010 was supervirulent on chicory leaf and on pea plantlet. This indicates that the genome of *E. chrysanthemi* also carries genes that might negatively influence the bacterial virulence.

The biological mechanisms involved in the interaction between a plant and a pathogenic bacterium are complex. This study confirms the important role of the bacterial envelope in a successful interaction with the plant. The study of the pathogenic behavior of *E. chrysanthemi* mutants on different hosts is the first step to understanding the molecular basis of host specificity. Although most of the Pin⁻ mutants seem to be affected in general mechanisms of virulence, some might be involved in host specificity. Moreover, some plant-inducible genes exert a negative control on the bacterial virulence and could contribute to restricting the host range.

ACKNOWLEDGMENTS

We thank P. Bechard, D. E. Egel, and W. Sachs for critical reading of the manuscript and G. P. Grondin for his assistance in the photographic work. This work was carried out under contract ECC BAP-01190-B of the Biotechnology Action Program. C. B. gratefully acknowledges the receipt of a postdoctoral fellowship from the Fonds pour la Formation des Chercheurs et l' Aide à la Recherche du Gouvernement du Québec.

LITERATURE CITED

Andro, T., Chambost, J. P., Kotoujansky, A., Cattaneo, J., Bertheau, Y., Barras, F., Van Gijsegem, F., and Coleno, A. 1984. Mutants of *Erwinia chrysanthemi* affected in the secretion of pectinases and cellulases. J. Bacteriol. 160:1199-1203.

Beaulieu, C., and Van Gijsegem, F. 1990. Identification of plant-inducible genes in *Erwinia chrysanthemi* strain 3937. J. Bacteriol. 172:1569-1575.

Bertheau, Y., Madjidi-Hervan, E., Kotoujansky, A., Nguyen-The, C., Andro, T., and Coleno, A. 1984. Detection of depolymerase isoenzymes after electrophoresis and electrofocusing, or in titration curves. Anal. Biochem. 139:383-389.

Boucher, C., Barberis, P., Trigalet, A., and Demery, D. 1985. Transposon mutagenesis of *Pseudomonas solanacearum*: Isolation of Tn*5*-induced mutants. J. Gen. Microbiol. 131:2449-2457.

Chang, A. C. Y., and Cohen, S. N. 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. 143:1141-1156.

Daniels, M. J., Dow, J. M., and Osbourn, A. E. 1988. Molecular genetics of pathogenicity in phytopathogenic bacteria. Annu. Rev. Phytopathol. 26:285-312.

Desmet, L., Faelen, M., Gamma, M.-J., Ferhat, A., and Toussaint, A. 1989. Characterization of amber mutations in bacteriophage Mu transposase: A functional analysis of the protein. Mol. Microbiol. 3:1145-1158.

Dickey, R. S. 1979. *Erwinia chrysanthemi*: A comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. Phytopathology 69:324-329.

Dickey, R. S. 1981. *Erwinia chrysanthemi*: Reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. Phytopathology 71:23-29.

Enard, C., Diolez, A., and Expert, D. 1988. Systemic virulence of *Erwinia chrysanthemi* 3937 requires a functional iron assimilation system. J. Bacteriol. 170:2419-2426.

Expert, D., and Toussaint, A. 1985. Bacteriocin-resistant mutants of *Erwinia chrysanthemi*: Possible involvement of iron acquisition in phytopathogenicity. J. Bacteriol. 163:221-227.

Glansdorff, N. 1965. Topography of cotransducible arginine mutations in *E. coli* K12. Genetics 51:167-169.

Hinton, J. C. D., Sidebotham, J. M., Hyman, L. J., Pérombelon, M. C. M., and Salmond, G. P. 1989. Isolation and characterization of transposon-induced mutants of *Erwinia carotovora* subsp. *atroseptica* exhibiting reduced virulence. Mol. Gen. Genet. 217:141-148.

Janse, J. D., and Ruissen, M. A. 1988. Characterization and classification of *Erwinia chrysanthemi* strains from several hosts in The Netherlands. Phytopathology 78:800-808.

Kotoujansky, A. 1987. Molecular genetics of pathogenesis by soft-rot Erwinias. Annu. Rev. Phytopathol. 25:405-430.

Lemattre, M., and Narcy, J. P. 1972. Une infection bactérienne nouvelle du Saintpaulia due à *Erwinia chrysanthemi*. C.R. Acad. Sci. 58:227-231.

Little, T. M., and Hills, F. J. 1978. Agricultural experimentation, design and analysis. John Wiley & Sons, New York.

Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Osbourn, A. E., Barber, C. E., and Daniels, J. 1987. Identification of plant-induced genes of the bacterial pathogen *Xanthomonas campestris* pv. *campestris* using a promotor-probe plasmid. EMBO J. 6:23-28.

Payne, J. H., Schoedel, C., Keen, N. T., and Collmer, A. 1987. Multiplication and virulence in plant tissue of *Escherichia coli* clones producing pectate lyase isoenzymes PLb and PLe at high levels and of *Erwinia chrysanthemi* mutant deficient in PLe. Appl. Environ. Microbiol. 53:2315-2320.

Pirhonen, M., Saarilahti, H., Karlsson, M.-B., and Tapio Palva, E. 1991. Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. Mol. Plant-Microbe Interact. 4:276-283.

Ratet, P., and Richaud, F. 1986. Construction and uses of a new transposable element whose insertion is able to produce gene fusions with the neomycin-phosphotransferase-coding region of Tn*903*. Gene 42:185-

192.
Resibois, A., Colet, M., Faelen, M., Schoojenans, E., and Toussaint, A. 1984. Phi EC-2, a new generalized transducting phage of *Erwinia chrysanthemi*. Virology 137:102-112.
Schoonejans, E., Expert, D., and Toussaint, A. 1987. Characterization and virulence of *Erwinia chrysanthemi* lipopolysaccharide-defective EC2-resistant mutants. J. Bacteriol. 169:4011-4017.
Van Gijsegem, F. 1986. Analysis of the pectin-degrading enzymes secreted by three strains of *Erwinia chrysanthemi*. J. Gen. Microbiol. 132:617-624.