Differential Regulation by Iron of *Erwinia chrysanthemi* Pectate Lyases: Pathogenicity of Iron Transport Regulatory (*cbr*) Mutants

Christel Sauvage and Dominique Expert

Laboratoire de Pathologie Végétale, Institut National Agronomique, 16 Rue Claude Bernard, 75231 Paris Cedex 05, France

Received 19 February 1993. Revision received 9 September 1993. Accepted 21 October 1993.

To incite systemic soft rot symptoms on African violets, *Erwinia chrysanthemi* 3937 requires a number of pectinolytic activities, including five pectate lyase isoenzymes (PLa to PLe) expressed from independent cistrons (*pelA* to *pelE*), and a functional high-affinity iron transport system mediated by chrysobactin. The low iron level in leaf intercellular fluids led us to consider this metal as a potential regulatory signal of PL production. This study shows that synthesis of PL activities is stimulated under low-iron conditions. The effect is independent of the presence of the catabolic inducer of *pel* gene expression and is mediated by the *cbrAB* operon, which negatively controls the transcription of iron transport genes in strain 3937. Study of transcriptional GUS fusions generated to each of the *pel* genes in various *cbr* backgrounds demonstrated that *cbrAB* positively controls the expression of *pelB*, *pelC*, and *pelE* under low iron. The pathogenicity of the wild-type strain on African violets was analyzed in relation to the iron status of the preinoculation growth medium and in comparison to that of a *cbr* mutant; wild-type cells appeared to be more aggressive in the first phase of the disease when grown under iron-deficient conditions before inoculation, but a delay was observed with the chrysobactin constitutive *cbr* mutant cells. This result is discussed in regard to the pleiotropic effect of the *cbr* mutation.

Additional keywords: pectinase, *Saintpaulia ionantha.*

Pectinolytic erwinias interact with a variety of host plants including vegetables (potato, chicory, etc.) and ornamentals (philodendron, carnation, orchid, etc.), giving rise in many cases to soft rot symptoms involving tissue maceration. Invasion of parenchymas results from colonization of intercellular spaces by bacterial cells and the subsequent release of several pectinolytic enzymes that enable the bacteria to dissolve cell walls, leading to rapid disorganization of the tissue (Garibaldi and Bateman 1971; Temsah *et al.* 1991). The symptom may spread throughout the plant, and systemic disease generally depends on the host-pathogen interaction as well as environmental factors such as temperature, moisture, and oxygen availability (Pérombelon and Kelman 1980; Pagel and Heitefuss 1990). In *Erwinia chrysanthemi* 3937, systemic development of bacterial cells inside its natural host African violets (*Saintpaulia ionantha* H. Wendl.) requires the integrity of their pectinolytic arsenal (Boccara *et al.* 1988; Boccara and Chatain 1989) mainly composed of one pectin methyl esterase and five pectate lyase isoenzymes (PLa to PLe) (Bertheau *et al.* 1984) as well as a functional high-affinity iron assimilation system (Enard *et al.* 1988) mediated by the siderophore chrysobactin (Persmark *et al.* 1989). Except for PLb and PLc activities, impairing any of the pectinolytic or iron transport functions by gene mutation results in reduced virulence on Afican violets; the bacteria are still able to colonize and to macrate the inoculated area to a variable extent, but they fail to invade the whole plant. A differential role for these redundant pectinolytic activities has long been a focus of attention on this and related erwinias (Collmer and Keen 1986). Their cytotoxic effect together with the fact that their substrates (pecate polymers and oligomers) are elicitors of plant defense reactions (Davies *et al.* 1986) may explain why the cognate genes that form independent cistrons (Reverchon *et al.* 1986; Tamaki *et al.* 1988) are subject to a panoply of regulatory signals (Collmer and Bateman 1981; Hugouvieux-Cotte-Pattat *et al.* 1986; Reverchon and Robert-Baudouy 1987; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1989). A sequential pattern for the expression of these diverse activities during pathogenesis is probably important, and in this regard, the study of Yang *et al.* (1992) on *E. carotovora* is informative. Several pectic derivatives identified as catabolic inducers of the pectinolytic pathway (Collmer and Bateman 1982; Nasser *et al.* 1991) have genes that are normally repressed by the transcriptional repressor KdgR (Condemine and Robert-Baudouy 1991; Reverchon *et al.* 1991). In addition, transcriptional activity of *pel* genes responds, to a variable extent, to catabolite repression, growth phase, temperature, and nitrogen starvation. Anaerobiosis stimulates *pelA*, *pelD*, and *pelE* genes, and high osmolarity increases expression of *pelE* (Hugouvieux-Cotte-Pattat *et al.* 1992).

The failure of chrysobactin iron-acquisition mutants to thrive *in planta* is primarily due to the fact that iron in leaf intercellular fluids is not readily available to bacterial cells. The iron level proved to be low enough to restrict the growth of mutants unable to produce or use chrysobactin and citrate, natural carriers of iron in higher plants that cannot be used by Erwinia cells (Expert and Gill 1991). Accordingly, the pro-

Corresponding author: D. Expert.

MPMI Vol. 7, No. 1, 1994, pp. 71-77

duction of chrysobactin was detected in leaf intercellular fluids of diseased plants (Neema *et al.* 1993). In this particular interaction, iron emerges as an environmental signal that triggers a set of functions required for the production of a siderophore and the ferric complex uptake system, which involves up to 25 loci (Franza *et al.* 1991), among them the *fct cbs* operon (Franza and Expert 1991). In bacterial cells, iron is detected at the molecular level by a two-factor system encoded by the *cbrAB* operon, which negatively controls the transcription of iron-regulated genes (Expert *et al.* 1992). These data led us to consider whether the iron content of the plant might not also influence the levels of pectinolytic activities, as previously shown in some bacterial pathogens of animals that specifically induce their toxins in low-iron environments (Calderwood and Mekalanos 1987; Poole and Braun 1988). In this work, we investigated this problem, studying the expression of *pel-uidA* gene fusions placed in various *cbr* genotypes. The pathogenic behavior of *cbr* mutants that constitutively express their iron-uptake systems was analyzed on African violets and compared with that of the wild-type strain derepressed for chrysobactin functions before inoculation. The data suggest that unbalanced production of the pectic isoenzymes, as observed in a *cbr* mutant when grown *in vitro* under iron-deficient conditions, changes the normal course of the disease.

RESULTS

Effect of iron on PL production in strain 3937 and in a *cbr* derivative.

We previously reported (Sauvage *et al.* 1990) that the production of PL in wild-type cells grown in the presence of polygalacturonate (PGA) was influenced by the availability of iron in the medium. The effect was related to PL synthesis. A mutant impaired in iron regulation was completely insensitive to iron fluctuations. In this study, we investigated whether iron might also influence the basal activity of the *pel* genes. PL activity was assayed in culture supernatant fluids, over time, during the late exponential and early stationary stages of bacterial growth (Fig. 1). In wild-type cells, concentrations of iron lower than 0.5 μM appeared to stimulate the PL basal level by a 10-fold factor, whereas no significant effect was apparent with the *cbr* mutants (Fig. 1).

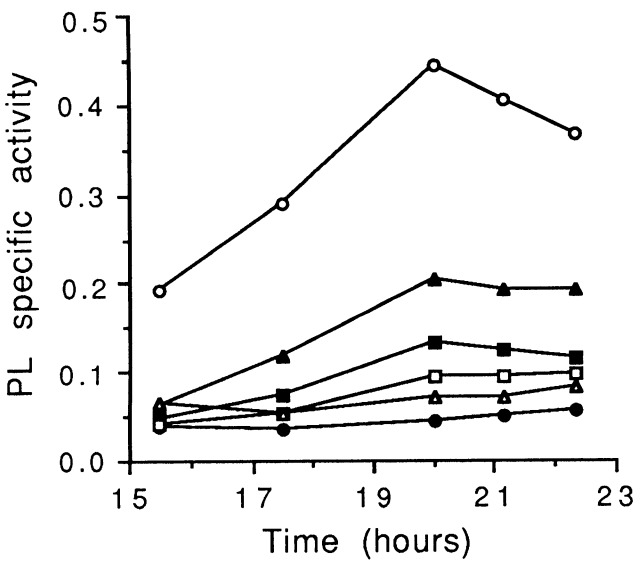

Fig. 1. Pectate-lyase (PL) activity in culture supernatant fluids from *Erwinia chrysanthemi* strain 3937 (circles) and its derivatives harboring the mutation *cbrA-145* (squares) or *cbrB-7* (triangles), grown under iron-rich (solid symbols) or iron-poor (open symbols) conditions in the absence of polygalacturonate.

Effect of iron on *pel* gene transcription in Cbr$^+$ and Cbr$^-$ cells.

The increase in PL production with decreasing iron might result from transcriptional control of *pel* gene expression mediated through the *cbrAB* operon. We checked this possibility by analyzing the expression of transcriptional GUS fusions generated with each of the five *pel* genes, in Cbr$^+$ and Cbr$^-$ phenotypes. For this purpose, the five *pel*::*uidA* fusions present in various genetic backgrounds were transduced in strain 3937 and in its derivative harboring the mutation *cbrB-7*. The mutation *cbrA-145* was introduced in the different *pel*::*uidA*-carrying strains by reverse genetics. GUS activity produced by these strains was recorded during their growth in iron-rich or iron-poor M63 medium. The study was simultaneously conducted under conditions of induction and repression of the *pel* genes. Since the effect of iron appeared to be independent of the presence of inducer, as previously observed for PL activity (see Fig. 2 for the *pelE::uidA* fusion as an example), only experiments performed in the absence of PGA are presented in detail (Fig. 2).

In Cbr$^+$ cells, a significant effect of iron on the expression of *pelB*, *pelC,* and *pelE* was seen. Iron depletion stimulated *pelB::* and *pelC::uidA* twofold; a threefold increase was apparent with *pelE::uidA*. In contrast, the *pelA* and *pelD* fusions were not stimulated by low-iron conditions. Globally, Cbr$^-$ cells were not stimulated by decreasing iron (Fig. 2), and the basal level of GUS activity in the *cbrA-145 pelD::uidA* mutant was even lower than that observed in the presence of iron for the *cbr* wild-type counterpart. No difference between the two *cbr* genotypes was detected (data not shown). The presence in Cbr$^-$ cells of the wild-type *cbr* locus on plasmid pDE9 could restore inducibility of the *pelE* fusion by iron as shown with the *pelE-uidA* fusion in Figure 3. Plasmid pDE6, which contains only *cbrA,* was inefficient (Fig. 3). Expression of *pelD* in the presence of pDE9 remained unchanged (data not shown). These results indicate that a functional *cbrAB* operon is required to promote regulation by iron of *pelB*, *pelC*, and *pelE*.

Pathogenicity of Cbr$^-$ mutants on African violets.

We examined the pathogenic behavior of Cbr$^-$ mutants (3937*cbrA-145* and 3937*cbrB-7*) on African violets by comparison with the wild-type strain cultured under iron-rich or iron-poor conditions, prior to inoculation. As presented in Figure 4, an increase in virulence was apparent when wild-type cells were grown under limited-iron conditions before inoculation; progression of the maceration symptom beyond the inoculated zone was accelerated. This was visualized through the relative numbers of phases 1, 2, and 3, as defined in Figure 4, during the first 72 hr after inoculation. Faster progression of the disease continued until 5 or 6 days postinoculation. Cbr$^-$ cells, which constitutively express chrysobactin and its transport system, proved to be less aggressive than the non-in-

duced wild-type cells (Fig. 5); a significant lag during the primary phases of the disease was evident. As shown with the mutant 3937*cbrA-145,* the delay appeared to be essential in the evolution of the disease; about half of the plants displaying delayed symptoms failed to give systemic responses. In the mutant strains, unlike the wild-type, iron conditions during growth prior to inoculation did not influence the first-phase spreading of disease (data not shown).

DISCUSSION

As a primary approach to the present work, we looked at the effect of various concentrations of iron on production of the diverse PL activities released during the growth of *E. chrysanthemi* cells. The stimulation of PL by iron deprivation presented in Figure 1, which was observed both in the presence (Sauvage *et al.* 1990) and absence of catabolic inducer, led us to analyze the expression of each of the *pel* genes independently under iron-rich and iron-poor conditions. In summary, our data show a threefold stimulation of the basal transcriptional activity of *pelB*, *pelC,* and *pelE* genes by low iron levels, an effect less than the 10-fold factor detected under the same conditions for the PL activity. Post-transcriptional effects resulting in varying translational levels might explain this difference. Alternatively, it is possible that PL cryptic activities, such as those of the exopolygalacturonate

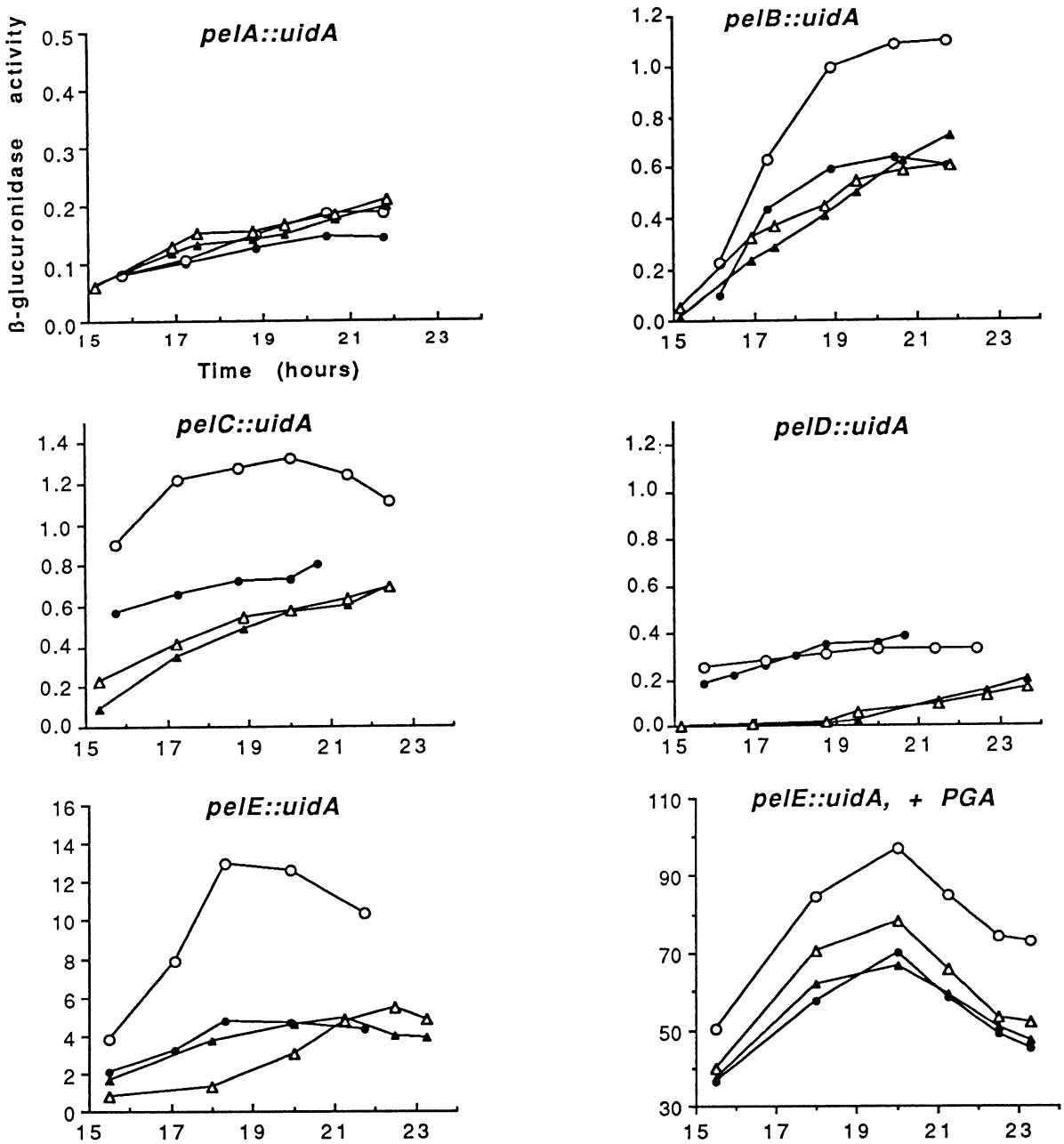

Fig. 2. Expression of chromosomal *pel::uidA* gene fusions in Cbr$^+$ (*Erwinia chrysanthemi* strain 3937, circles) and Cbr$^-$ (strain 3937 *cbrA-145*, triangles) cells grown under iron-rich (solid symbols) and iron-poor conditions (open symbols). As an example, the graph *pelE::uidA* + polygalacturonate (PGA) shows the expression of the fusion in cells grown in the presence of inducer.

lyase of *E. chrysanthemi* EC16 (Brooks *et al.* 1990), might be inducible by low-iron conditions. The regulatory process by iron appeared to be mediated through the *cbrAB* operon. Since in the absence of functional Cbr products, the *pel* gene transcriptional activity corresponds to the iron-repressed level in wild-type strain, we are tempted to conclude that *cbr* behaves as an activator. Thus, the *cbrAB* operon does not only control the iron transport functions per se but also major critical factors for the virulence such as PL. Interpretation of this result at the molecular level would be speculative. So far, we do not know how the *cbr*-encoded products CbrA and CbrB operate to activate the switch of the chrysobactin operon in response to iron (Expert *et al.* 1992). How do these proteins proceed, and how do they interact to guarantee iron-sensing and DNA-binding activities? Such questions are also relevant for *pel* gene activation. Besides the KdgR consensus operator sequence, which has been identified in all *pel* gene promoting regions, additional regulatory signals were observed upstream from the translational start of *pelB* and *pelC* (Tamaki *et al.* 1988; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1992). These might be the target for any other effector responsive to an environmental signal. It seems likely that several regulatory proteins interact in a competitive or cooperative fashion with DNA-recognition sequences, thus resulting in modulation to a variable degree of activities that are critical for the pathogenic cycle.

In this regard, the pathogenicity of *cbr* mutants is interesting. Indeed, from the assay performed with wild-type cells induced for their chrysobactin system prior to inoculation, we expected that the constitutive expression of the chrysobactin system linked to the *cbr* mutation would be responsible for accelerated symptom development. The kinetics of chrysobactin production after inoculation reveals a significant influence on the pathogenesis process. However, the mutants are less aggressive than the parent, and the effect is particularly significant because it occurs at an early phase of the disease, when bacteria are spreading beyond the inoculated zone. The delay at this point is probably critical to the final outcome of the disease, as subsequently observed in the slower progression of symptoms. It should be noted that the growth of *cbr* mutant cells under high-iron conditions was the same as that of parental cells and was accelerated during the early exponential stage under low-iron conditions (data not shown). In contrast, when no delay occurred, the ultimate phase of the disease was sometimes enhanced. It seems, therefore, that the qualitative and quantitative differences in the pectinolytic activity of the mutants might determine their pathogenic behavior; the lack of stimulation of *pelB*, *pelC*, and *pelE* even at a weak level by low iron levels, a condition which prevails in the intercellular environment (Neema *et al.* 1993), may create a disadvantage for the bacteria when they begin to spread within the leaf. It is noteworthy that the effect concerns PLb and PLc activities, which are not absolutely required for virulence on African violets, and the PLe isoenzyme, which, in contrast, is essential for symptom spreading. In addition, the very low *pelD* transcriptional expression found

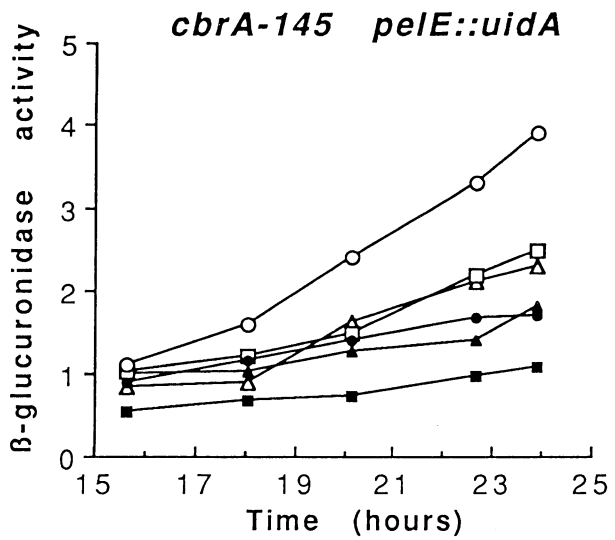

Fig. 3. Expression of the *pelE::uidA* fusion in *Erwinia chrysanthemi* strain 3937 *cbrA-145* harboring the wild-type locus *cbrA* or both *cbrA* and *cbrB* expressed on plasmid pRK767. Cells were grown under iron-rich (solid symbols) and iron-poor conditions (open symbols). Circles correspond to the whole locus (pDE6), squares to *cbrA* (pDE9), and triangles to the vector alone.

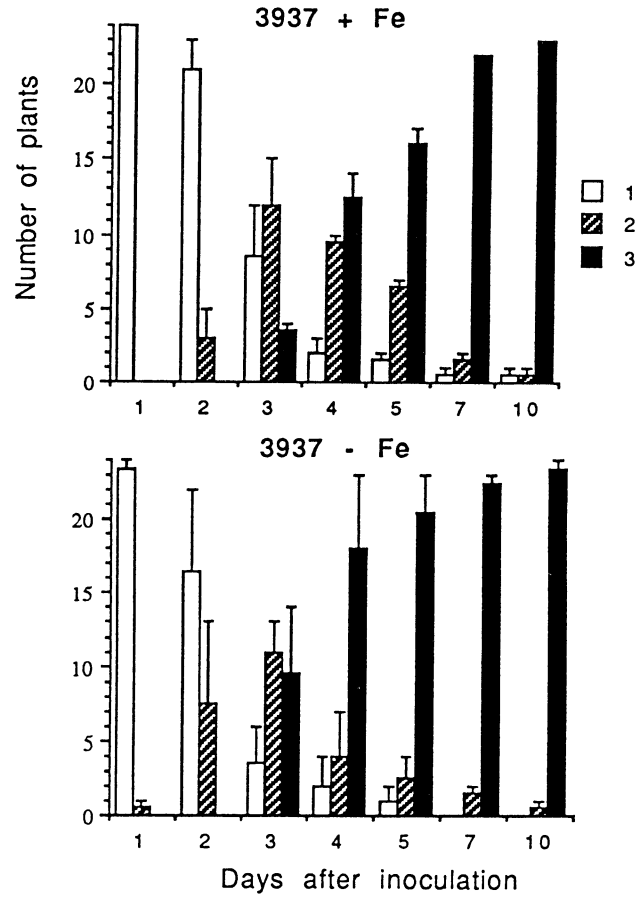

Fig. 4. Evolution of symptoms induced by *Erwinia chrysanthemi* strain 3937 on African violets, when grown under iron-rich (+Fe) or iron-poor conditions (–Fe) prior to inoculation. Procedures are detailed in Materials and Methods. The significant spreading phases of the disease were scored as follows: 1 = maceration localized at the inoculated area, 2 = maceration covering the inoculated leaf, 3 = maceration invading the petiole, 4 = spreading of the maceration towards other aerial parts of the plant. In these assays, unlike those reported in Figure 5, no phase 4 symptoms were observed. Bar heights correspond to the mean values obtained for each disease severity level during three independent assays. Standard deviations are indicated.

in *cbr* mutants is, in this context, relevant. Like other bacterial pathogens, including species pathogenic to animals (Wick *et al.* 1991), the pathogenic behavior of strain 3937 on its host plant resides in a multifunctional process that must be tightly controlled at the molecular level. In the present case, synthesis of siderophores and the cognate transport system is energy-consuming, and it is possible that activation of several catabolic genes under conditions of iron starvation correlates with the requirement of additional substrates. *In planta* expression of *pel* gene fusions as well as chrysobactin operon fusions is now under investigation.

The conservation through evolution of so many genes that seem to encode redundant enzymatic activities may not have occurred randomly. Selective pressure may have been applied at the regulatory level, and differential expression of the pectate lyase genes by iron or any other signal identified previously can reflect some adaptive response to a given host and/or to its degree of differentiation. The versatility conferred by this pectinolytic redundance may explain why this species can infect a large range of plants to variable degree. As a full member of the family of the Enterobacteriaceae, *E. chrysanthemi* 3937, through regulation by iron (Bullen *et al.* 1991), demonstrates adaptive features typical of its enteric relatives.

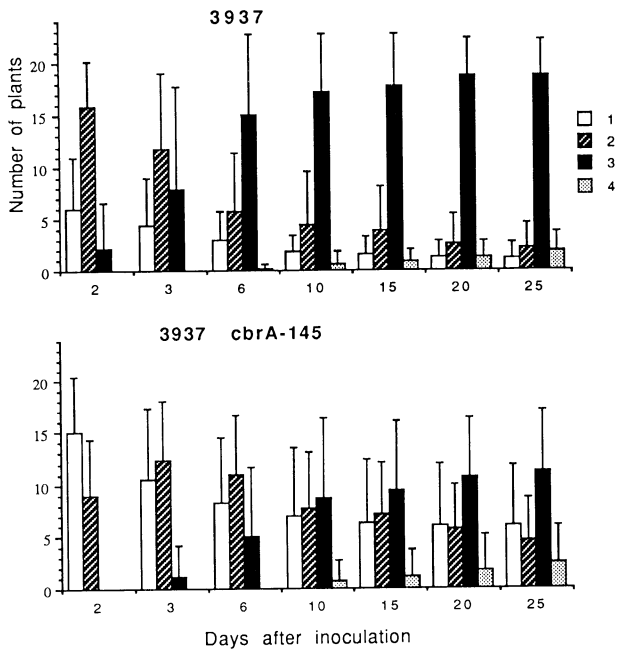

Fig. 5. Evolution of symptoms induced by *Erwinia chrysanthemi* strain 3937 and its mutant derivative harboring the mutation *cbrA-145* on African violets. Behavior of the strain harboring the mutation *cbrB-7* was the same as 3937 *cbrA-145* (data not shown). Bar heights correspond to the mean values obtained for each disease severity level defined in Figure 4 in the course of five independent assays as described in Materials and Methods.

MATERIALS AND METHODS

Strains and media.

The bacterial strains, plasmids, and bacteriophage used are described in Table 1. The enriched medium used was L broth (Miller 1972). M63 minimal medium was treated with 8-hydroxyquinoline to remove contaminating iron (Expert and Toussaint 1985) and was supplemented with 10 μM $FeCl_3$ to provide iron-rich conditions. Glycerol (2 g/L) was used as a carbon source, and PGA (4 g/L) was used as a pectate lyase inducer. Media were solidified with Difco agar (12 or 15 g/L for plates and 7 g/L for soft overlays). Antibacterial agents were used as reported previously (Franza *et al.* 1991).

Construction of bacterial strains.

The *pelA*::, *pelB*::, *pelC*::, *pelD*::, and *pelE*::*uidA* fusions were transduced in strain 3937 with phage PhiEC2, as described previously (Franza *et al.* 1991), using kanamycin resistance as a selective marker. The phenotype of recipient strains with regard to production of pectate lyase isoenzymes was controlled by electrofocusing in ultrathin polyacrylamide gels as described previously (Bertheau *et al.* 1984). The *cbrB-7* mutation was then introduced by transduction, using chloramphenicol as a selective marker. To introduce the *cbrA-145* mutation, plasmid pDE4, which contains the mutagenized insert, was transferred by transformation to the GUS fusion–harboring strains as described by Expert *et al.* (1992). Exchange recombination of the plasmid DNA insert with the chromosome was monitored as described before (Roeder and Collmer 1985). In all cases, the Cbr^- phenotype was checked on CAS agar medium (Schwyn and Neilands 1987) as indi-

Table 1. Bacterial strains, plasmids, and bacteriophage used in this study

	Characteristics	Reference or source
Erwinia chrysanthemi		
3937	Wild-type strain isolated from *Saintpaulia ionantha*	Kotoujansky *et al.* 1982
3937 *cbrB-7*	*cbr-7*: with a MudIIpR13 insertion in *cbrAB*, Cbr^-, Cm^R, Lac^-	Expert *et al.* 1992
L37 *cbrA-145*	*cbr-145*: with a MudIIpR13 insertion in *cbrAB*, Cbr^-, Cm^R, Lac^-	Expert *et al.* 1992
A1888	*PelA*::*uidA*, Km^R	N. Hugouvieux-Cotte-Pattat
A1732	*arg-10*, *pelB*::*uidA*, Km^R	N. Hugouvieux-Cotte-Pattat
A1880	*pelC*::*uidA*, Km^R	N. Hugouvieux-Cotte-Pattat
A1798	*pelD*::*uidA*, Km^R	N. Hugouvieux-Cotte-Pattat
A1828	*pelE*::*uidA*, Km^R	N. Hugouvieux-Cotte-Pattat
Escherichia coli		
C600	F^- *thi-1 thr-1 leuB6 lacY1 tonA21 supE44* $lambda^-$	E. Schoonejans
Plasmids		
pRK2013	Km	Ditta *et al.* 1980
pDE9	pRK767 with a 1.8-kb *Bam*HI insert containing *cbrA* (Tc)	Expert *et al.* 1992
pDE6	pRK767 with an 8-kb *Hin*dIII insert containing *cbrAB* (Tc)	Expert *et al.* 1992
pDE4 *cbrA-145*	pUC18 with a 12-kb *Eco*RI insert containing *cbrA-145* (Ap, Cm)	Expert *et al.* 1992
Phage		
PhiEC2	General transducing phage of *E. chrysanthemi*	Résibois *et al.* 1984

cated previously (Expert *et al.* 1992). Plasmids pDE6 and pDE9 were introduced in the *cbr pel::uidA* derivatives by conjugation, using pRK2013 as a helper plasmid, as described previously (Franza *et al.* 1991).

Enzyme activity assays.

Bacterial cells were first grown in iron-rich M63 medium for 8 hr, then diluted in iron-poor M63 medium (iron removed) either supplemented with $FeCl_3$ or not, in the presence or absence of PGA, to give an optical density at 600 nm of 0.003. Cultures were incubated with shaking for 25 hr. Samples were collected periodically and stored at –20° C for the β-glucuronidase assay. PL activity was assayed in the supernatant fluids kept at –20° C. This activity was determined by the release from PGA of unsaturated products absorbing at 235 nm (Moran *et al.* 1968) in 0.1 M Tris buffer, pH 8.5, and 0.5 mM $CaCl_2$. One unit of PL activity was defined as 1 μM unsaturated products liberated per minute, per milligram of bacterial dry weight. β-glucuronidase activity was assayed, using *p*-nitrophenyl-β-D-glucuronide as substrate. The degradation product, *p*-nitrophenol, was detected at 405 nm (Novel *et al.* 1974). Specific activity was expressed in nanomoles of *p*-nitrophenol liberated per minute per unit of optical density at 600 nm. Given that enzymatic activities differ throughout independent assays, the whole experiment was performed at least four times, once for each strain and condition. The results were analyzed statistically, using a two-tailed *t* test at the 5% level of significance.

Pathogenicity assay.

Pathogenicity was tested on potted African violets as described (Expert and Toussaint 1985), with modifications; wild-type cells were grown in iron-rich or iron-poor M63 medium containing glycerol as a carbon source, until reaching an optical density at 600 nm of 0.7. Cultures were filtered through a Millipore membrane (pore size, 0.45 μm), and bacterial cells retained on the filter were suspended in a volume of NaCl solution (9 g/L) to give an optical density of 0.35. The inoculum was 100 μl of the resulting suspension. The pathogenicity of *cbr* mutant cells was compared with that of the wild-type strain by inoculating 100 μl of a twofold dilution of a bacterial culture grown in iron-rich M63 medium until it reached an optical density of 0.7. Plants were maintained in a growth chamber at 25–30° C under high moisture conditions, with 16 hr under illumination and 8 hr darkness, daily. Progression of the symptoms was scored daily, for 15 or 25 days. An index scale from 1 to 4 indicating the disease severity was used. In each assay, 24 plants were inoculated by strain or condition. Assays were carried out in triplicate for testing the effect of the preinoculation medium on pathogenicity of wild-type strain, and five times for the pathogenicity of the *cbr* mutant as compared to wild-type strain.

ACKNOWLEDGMENTS

We thank the *Erwinia* group from Lyon for providing us with the *pel::uidA* constructions and for their interest in our work, Marc Girondot and Jacqueline Laurent for their help on statistical analyses, and the reviewers of MPMI for their stimulating comments. This work was supported by the Institut National de la Recherche Agronomique (INRA). C. S. was supported by the Ministère de la Recherche et de l'Enseignement Supérieur. D. E. is a researcher from the Centre National de la Recherche Scientifique (CNRS).

LITERATURE CITED

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

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 of saintpaulia plants. Physiol. Mol. Plant Pathol. 3:95-104.

Brooks, A. D., Yang He, S., Gold, S., Keen, N. T., Collmer, A., and Hutcheson, S. T. 1990. Molecular cloning of the structural gene for exopolygalacturonate lyase from *Erwinia chrysanthemi* EC16 and characterization of the enzyme produced. J. Bacteriol. 172:6950-6958.

Bullen, J. J., Ward, C. G., and Rogers, H. J. 1991. The critical role of iron in some clinical infections. Eur. J. Clin. Microbiol. Infect. Dis. 10:613-617.

Calderwood, S. T., and Mekalanos, J. J. 1987. Iron regulation of shiga-like toxin expression in *Escherichia coli* is mediated by the *fur* locus. J. Bacteriol. 169:4759-4764.

Collmer, A., and Bateman, D. 1981. Impaired induction and self-catabolite repression of extracellular pectate lyase in *Erwinia chrysanthemi* muants deficient in oligo-galacturonide lyase. Proc. Natl. Acad. Sci. USA 78:3920-3924.

Collmer, A., and Bateman, D. 1982. Regulation of extracellular pectate lyase in *Erwinia chrysanthemi*: Evidence that reaction products of pectate lyase and exo-poly-D-galacturonase mediate induction on galacturonan. Physiol. Plant Pathol. 21:127-139

Collmer, A., and Keen, N. T. 1986. The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. 24:383-409.

Condemine, G., and Robert-Baudouy J. 1991. Analysis of an *Erwinia chrysanthemi* gene cluster involved in pectin degradation. Mol. Microbiol. 5:2191-2202.

Davies, K. R., Lyon, G. D., Albersheim, P., and Dell, A. 1986. Host-pathogen interactions. XXV. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 73:52-60.

Ditta, G., Stanfield, S., Corbin, D., and Helinsky, D. R. 1980. Broad host range DNA cloning system for gram-negative bacteria: Construction of a gene bank of *Rhizobium meliloti*. Proc. Natl. Acad. Sci. USA 77:7347-7351.

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 Gill, P. R. 1991. Iron: A modulator in bacterial virulence and symbiotic nitrogen-fixation. Pages 229-245 in: Molecular Signals in Plant-Microbe Communications. D. P. S. Verma, ed. CRC Press, Boca Raton, FL.

Expert, D., Sauvage, C., and Neilands J. B. 1992. Negative transcriptional control of iron transport in *Erwinia chrysanthemi* involves an iron responsive two-factor system. Mol. Microbiol. 6:2009-2017.

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

Franza, T., Enard, C., Van Gijsegem, F., and Expert, D. 1991. Genetic analysis of the *Erwinia chrysanthemi* 3937 chrysobactin iron transport system: Characterization of a gene cluster involved in uptake and biosynthetic pathway. Mol. Microbiol. 5:1319-1329.

Franza, T., and Expert, D. 1991. The virulence-associated chrysobactin iron uptake system of *Erwinia chrysanthemi* 3937 involves an operon encoding transport and biosynthetic functions. J. Bacteriol. 173:6874-6881.

Garibaldi, A., and Bateman, D. F. 1971. Pectic enzymes produced by *Erwinia chrysanthemi* and their effects on plant tissue. Physiol. Plant Pathol. 1:25-40.

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

Hugouvieux-Cotte-Pattat, N., Reverchon, S., Condemine, G., and Robert-Baudouy, J. 1986. Regulatory mutants affecting the synthesis of pectate lyases in *Erwinia chrysanthemi*. J. Gen. Microbiol. 132:2099-2106.

Hugouvieux-Cotte-Pattat, N., and Robert-Baudouy, J. 1989. Isolation of *Erwinia chrysanthemi* mutants altered in pectinolytic enzyme production. Mol. Microbiol. 3:1587-1597.

Hugouvieux-Cotte-Pattat, N., and Robert-Baudouy J. 1992. Analysis of the regulation of the *pelB,C* genes in *E. chrysanthemi* 3937. Mol. Microbiol. 6:2363-2376.

Kotoujansky, A., Lemattre, M., and Boistard, P. 1982. Utilization of a thermosensitive plasmid bearing transposon Tn*10* to isolate Hfr donor strains of *Erwinia carotovora* subsp. *chrysanthemi*. J. Bacteriol. 150:122-131.

Moran, F., Nasumo, S., and Starr M. P. 1968. Extracellular and intracellular polygalacturonic acid trans-eliminase of *Erwinia carotovora*. Arch. Biochem. Biophys. 123:298-306.

Miller, J. F. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

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

Neema, C., Laulhère, J. P., and Expert, D. 1993. Iron deficiency induced by chrysobactin in saintpaulia leaves inoculated with *Erwinia chrysanthemi*. Plant Physiol. 102:967-973.

Novel, G., Didier-Fichet, M. L., and Stoeber, F. 1974. Inducibility of β-glucuronidase in wild type and hexuronate-negative mutants of *Escherichia coli* K-12. J. Bacteriol. 120:89-95.

Pagel, W., and Heitefuss, R. 1990. Enzyme activities in soft-rot pathogenesis of potato tubers: Effects of calcium, pH, and degree of pectin esterification on activities of polygalacturonase and pectate lyase. Physiol. Mol. Plant Pathol. 37:9-25.

Pérombelon, C. M., and Kelman, A. 1980. Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. 18:361-387.

Persmark, M., Expert, D., and Neilands, J. B. 1989. Isolation, characterization and synthesis of chrysobactin, a compound with siderophore activity from *Erwinia chrysanthemi*. J. Biol. Chem. 264:3187-3193.

Poole, K., and Braun, V. 1988. Iron regulation of *Serratia marcescens* hemolysin gene expression. Infect. Immun. 56:2667-2671

Résibois, A., Colet, M., Faelen, M., Schoonejans, E., and Toussaint, A. 1984. Phi-EC2 a new generalized transducing phage of *Erwinia chrysanthemi*. Virology 137:102-112.

Reverchon, S., and Robert-Baudouy, J. 1987. Regulation of expression of pectate lyase genes *pelA, pelD, pelE* in *Erwinia chrysanthemi*. J. Bacteriol. 169:2417-2423.

Reverchon, S., Van Gijsegem, F., Rouve, M., Kotoujansky, A., and Robert-Baudouy, J. 1986. Organisation of a pectate lyase gene family in *Erwinia chrysanthemi*. Gene 29:216-224.

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.

Roeder, D. L., and Collmer, A. 1985. Marker-exchange mutagenesis of a pectate lyase isozyme gene in *Erwinia chrysanthemi*. J. Bacteriol. 164:51-56.

Sauvage, C., Franza, T., and Expert, D. 1990. Iron as a modulator of pathogenicity of *Erwinia chrysanthemi* on *Saintpaulia ionantha*. Pages 94-97 in: Advances in Molecular Genetics of Plant-Microbe Interactions. Vol 1. H. Hennecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Amsterdam.

Schwyn, B., and Neilands, J. B. 1987. Universal chemical assay for the detection and and determination of siderophores. Anal. Biochem. 160:47-56.

Tamaki, S. J., Gold, S., Robeson, M., Manulis, S., and Keen, N. T. 1988. Structure and organisation of the *pel* genes from *Erwinia chrysanthemi* EC16. J. Bacteriol. 170:3468-3478.

Temsah, M., Bertheau, Y., and Vian, B. 1991. Pectate lyase fixation and pectate disorganization visualized by immunocytochemistry in *Saintpaulia ionantha* infected by *Erwinia chrysanthemi*. Cell Biol. Int. Rep. 15:611-620.

Wick, M. J., Madara, J. L., Fields, B. N., and Normark, S. J. 1991. Molecular cross talk between epithelial cells and pathogenic microorganisms. Cell 67:651-659.

Yang, Z., Cramer, C. L., and Lacy, G. H. 1992. *Erwinia carotovora* subsp. *carotovora* pectic enzymes: *In planta* gene activation and roles in soft-rot pathogenesis. Mol. Plant-Microbe Interact. 5:104-112.