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The PecT Repressor Coregulates Synthesis of Exopolysaccharides and Virulence Factors in *Erwinia chrysanthemi*

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***Erwinia chrysanthemi* 3937 synthesizes an exopolysaccharide (EPS) composed of rhamnose, galactose, and galacturonic acid. Fourteen transcriptional fusions in genes required for EPS synthesis, named *eps*, were obtained by Tn*5-B21* mutagenesis. Eleven of them are clustered on the chromosome and are repressed by PecT, a regulator of pectate lyase synthesis. In addition, expression of these fusions is repressed by the catabolite regulatory protein, CRP, and induced in low osmolarity medium. The three other mutations are located in genes that are not regulated by *pecT*. A 13-kb DNA fragment containing *pecT*-regulated *eps* genes has been cloned. All the genes identified on this fragment are transcribed in the same orientation and could form a large operon. The promoter region of this operon has been sequenced. It contains a JUMPstart sequence, a sequence required for the expression of polysaccharide-associated operons. *E. chrysanthemi* 3937 produces a systemic soft rot on its host *Saintpaulia ionantha*. An *eps* mutant was less efficient than the wild-type strain in initiating a maceration symptom, suggesting that production of EPS is required for the full expression of the *E. chrysanthemi* virulence.**

Synthesis of exopolysaccharides (EPS) is an important factor for many plant-pathogenic bacteria to provoke normal disease symptoms such as water-soaking or wilting. Their role has been well documented in bacteria such as *Ralstonia solanacearum*, *Erwinia stewartii* (*Pantoea stewartii* subsp. *stewartii*), and *Erwinia amylovora* (Denny 1995; Leigh and Coplin 1992). Regulation of their synthesis is often complex and responds to numerous environmental and specific signals. *R. solanacearum* causes a lethal wilting on many plant species. Production of an acidic, glucosamine-rich EPS is one of the main causes of its pathogenicity (Denny and Baek 1991). Besides a specific regulation by two two-component regulatory systems (VsrA/D and VsrB/C) and the activator XpsR (Huang et al. 1995), synthesis of this EPS is coregulated with that of other virulence factors such as endopolygalacturonase A, a pectin methylesterase, and an endoglucanase, through a regulatory cascade that involves the products of the *phcA*, *B*, *R*, and *S* genes (Brumbley et al. 1993; Clough et al. 1997).

EPS are the main virulence factors of *E. stewartii* and *E. amylovora*. *E. stewartii* produces stewartan, an extracellular heteropolysaccharide that contributes to the water-soaking symptoms observed in Stewart's wilt of corn. *E. amylovora* synthesizes two types of EPS, levan and amylovoran. In these two bacteria, regulation of EPS synthesis is very similar to that described for the regulation of colanic acid capsule synthesis in *Escherichia coli*, where the *cps* genes are activated by *rcsA* and the *rcsB-rcsC* couple. The quantity of the regulatory protein RcsA in *E. coli* and *E. amylovora* is controlled by the Lon protease. RcsB is the regulatory part of a two-component system that is negatively regulated by the membrane sensor RcsC on the level of phosphorylation (Gottesman 1995). In *E. stewartii*, a negative regulator, EsaR, mediates quorum-sensing control of stewartan synthesis (Beck von Bodman and Farrand 1995; Beck von Bodman et al. 1998). Nothing has been described regarding the possible role of EPS in the pathogenicity of pectinolytic Erwinias.

Production of plant cell wall-degrading enzymes, mostly pectinases, is the main cause of *E. chrysanthemi* pathogenicity. The synthesis of virulence factors is controlled in *E. chrysanthemi* by three main specific repressors: KdgR, PecS, and PecT. KdgR regulates the genes of the pectin degradation pathway and the genes allowing the secretion of pectinases in response to the presence of pectin metabolism products (Condemine and Robert-Baudouy 1987b; Nasser et al. 1994). PecS is a repressor of the pectate lyase genes, of the secretion pathway genes, and of genes required for the synthesis of a blue pigment (Reverchon et al. 1994). The signal to which it responds is, for the moment, unknown. A *pecT* mutant synthesizes pectate lyases at a higher level but exhibits additional phenotypes: it flocculates when grown in liquid minimal medium, indicative of a modification of cell surface properties, and the colonies have a mucoid aspect on minimal medium agar plate (Surgey et al. 1996). This is a typical sign of an increased synthesis of EPS. An analysis of the EPS produced by *E. chrysanthemi* 3937 showed that it is composed of L-rhamnose, D-galactose, and galacturonic acid (4:1:1) (R. Montgomery, *personal communication*). To establish the coregulation of virulence factors and EPS synthesis genes in *E.*

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chrysanthemi, we have studied the regulation of EPS synthesis by constructing gene fusions in the *eps* genes, involved in EPS synthesis. We have shown that most of the *eps* genes are repressed by PecT and the catabolite regulatory protein, CRP. Moreover, we have studied the pathogenicity of an EPS-deficient strain.

RESULTS

Isolation of EPS-deficient mutants.

On 0.2% glycerol-containing minimal medium agar plates *E. chrysanthemi pecT* mutants have a mucoid aspect that is more pronounced when the medium is supplemented with 1% glucose. The EPS overproducing *pecT* mutant A2148 was mutagenized with the transposon Tn*5-B21*. This transposon, which encodes tetracycline resistance, also contains at one of its extremities the *lacZ* gene deleted of its regulatory regions. This allows the construction of a transcriptional fusion with the gene in which the transposon is inserted when it is in the proper orientation. Nonmucoid mutants were obtained at a frequency of 1%. Of the 34 nonmucoid mutants obtained, 14 had a Lac^+ phenotype, indicating that the transposon was inserted in the proper orientation to give a transcriptional fusion. When the insertion present in these 14 strains was transduced into a wild-type background (strain A350), the transductants were EPS^-. The expression of the fusions in the wild-type and *pecT* backgrounds was compared. Eleven fusions had a higher expression in the *pecT* background, while three (*eps-21*, *eps-22*, and *eps-23*) were expressed at the same level in both backgrounds (data not shown). A strain containing a *pecT*-regulated insertion (strain A2575) was retained for further investigation.

The transposon inserted in strain A2575 was mapped between the *gal-1* and *ade-3* markers on the *E. chrysanthemi* chromosomal map (Hugouvieux-Cotte-Pattat et al. 1989). *pecT* mutants flocculate when grown in liquid minimal medium. EPS^- mutants retained this capacity, indicating that overproduction of EPS is not the cause of flocculation. The level of pectate lyase synthesis was not changed in EPS^- mutants (data not shown).

Cloning of the EPS synthesis genes and construction of a *uidA* fusion.

Tn*5-B21* insertions are not very stable in *E. chrysanthemi* and often give secondary transpositions. We constructed a stable gene fusion by inserting a *uidA*-kan cassette into the *eps* locus mutated in strain A2575. The chromosomal DNA of strain A2575 was extracted, digested with the enzyme *Nsi*I, which does not cut Tn*5-B21*, and ligated into *Pst*I-digested pBluescript. Selection of Tet^r clones allowed the isolation of a plasmid containing a 11.2-kb DNA fragment, including 8 kb of Tn*5-B21* DNA. The remainder, 3.2 kb, corresponded to chromosomal DNA adjacent to the point of insertion. A rough restriction map of this plasmid indicated that it contained a unique *Bgl*II site 1.4 kb upstream of the *lacZ* extremity of Tn*5-B21*. A *uidA*-kan cassette was inserted into this site in both orientations. The insertion that created a transcriptional fusion with *uidA* (Fig. 1) was recombined into the *E. chrysanthemi* chromosome. The recombinant strain (A2952) was not mucoid on 1% glucose plates, indicating that the *Bgl*II site is inside an EPS synthesis gene. The Kan^r insertion of A2952 was transduced into the 14 Tn*5-B21* insertions and the percentage of Tet^s transductants was determined. The *uidA*-kan insertion strongly cotransduced (>90%) with the 11 *pecT*-regulated Tn*5-B21* insertions but not with the three non *pecT*-regulated insertions, indicating that the 11 *pecT*-regulated insertions are clustered on the chromosome.

EPS synthesis genes are usually grouped in a large operon. To clone additional *eps* genes, we digested the chromosomal DNA of strain A2952 by the restriction enzymes *Sal*I or *Hin*dIII, ligated the fragments in pBluescript, and selected Kan^r clones. Analysis of these plasmids showed that we have cloned 6.5 kb of DNA located upstream of the point of insertion of the *uidA*-kan cassette and 7 kb downstream. Partial sequencing was performed, using a number of evenly spaced restriction sites (Fig. 1). The six open reading frames (ORFs) deduced from sequenced fragments from the *Sal*I1, *Pst*I1, *Nsi*I2, *Bgl*II2, *Bam*HI2, and *Pst*I2 sites showed homology with genes present in data bases (Table 1). Five of them, oriented in the same direction, had homology with EPS synthesis genes present in various bacteria; using the nomenclature recommended in Reeves et al. (1996): *wza, wzc, weaP, rmlB,* and *rmlA* (Fig. 1). The gene located upstream of *wza*, ORF1, is in the opposite orientation, and does not present homology with known EPS synthesis genes. Sequencing performed from the *Hin*dIII site did not yield significant homology with any sequence present in data bases.

Regulation of the EPS synthesis genes.

The insertion of strain A2952 is in the *weaP* gene. This *weaP*::*uidA* fusion was used to study the factors regulating

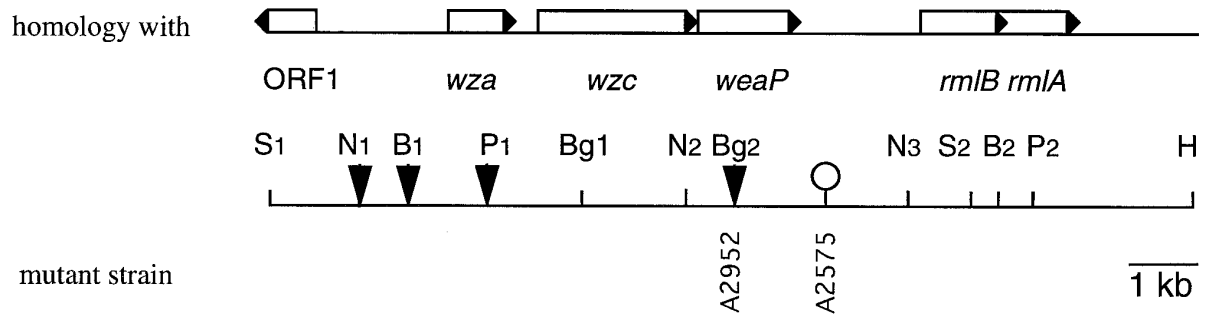

Fig. 1. Physical and genetic map of a 13-kb DNA fragment containing exopolysaccharide (EPS) synthesis genes. Bottom line indicates position of restriction sites. S: *Sal*I, N: *Nsi*I, B: *Bam*HI, P: *Pst*I, Bg: *Bgl*II, H: *Hin*dIII. Position of insertion of Tn*5-B21* in strain A2575, indicated by an open circle. Position of insertion of *uidA*-kan cassettes, indicated by a black triangle. Position of genes deduced from partial DNA sequencing, indicated on top line.

EPS synthesis. Anaerobiosis or high osmolarity did not modify expression of the fusion. Growth in a medium of low osmolarity (50 mOsm) induced threefold greater expression (Table 2). Incubation of the plates at 37°C increased EPS production and this effect was confirmed by assay of the fusion expression. Addition of leucine, which prevents flocculation due to the *pecT* mutation, did not modify the expression of the *weaP* gene. The effect of various carbon sources as potential inducers was tested. Polygalacturonate, glucuronate, plant extract, galacturonate, and galactose (the last two sugars are components of *E. chrysanthemi* 3937 EPS) had no effect. In contrast, addition of rhamnose (another sugar present in EPS) or glucose at high concentration (1%) increased expression of the fusion 1.5- to twofold (Table 2).

The effect of the genes regulating pectinase synthesis was tested. As expected from the above results, the expression of the fusion was increased (threefold) in a *pecT* mutant (Table 2). Culture of the *pecT weaP*::*uidA* strain with glucose as the carbon source led to a further increase, suggesting that PecT is not the regulator that senses glucose concentration. Induction by low osmolarity was higher (sixfold) in a *pecT* than in the wild-type background. In a *pecS* mutant, expression of the *eps* gene was lower, whatever the culture conditions. Expression of the fusion was not significantly modified in a *kdgR* background. To test if the glucose effect could be mediated by the catabolite regulatory protein, CRP, the fusion was introduced into a *crp* background. Its expression increased more than 10-fold (Table 2). Thus, CRP is a repressor of EPS synthesis. This effect was cumulative with that of the *pecT* mutation (Table 2). Recently, Missiakas and Raina (1997) have shown that the periplasmic stress caused by a *dsbA* mutation (no formation of disulfide bonds in periplasmic proteins) was able to induce production of colanic acid capsular polysaccharide in *E. coli*. In *E. chrysanthemi*, a *dsbA* mutation had a similar effect on EPS gene expression (Table 2).

Analysis of the *eps* gene regulatory region.

A *uidA*-kan cassette extracted from plasmid pUIDK3 was inserted into the *Nsi*I1 and *Bam*HI1 sites present upstream of *wza* and in the *Pst*I1 site present in *wza* (Fig. 1). This cassette was constructed so that transcription occurs from the kanamycin resistance gene into the downstream DNA. Insertion into the *Pst*I1 site with the orientation of *wza* gave rise to an EPS$^-$ mutant in which expression of β-glucuronidase was regulated by *pecT* and *crp* (data not shown). Insertion into the *Bam*HI1 site in the orientation of *wza* gave an EPS$^+$ strain and expression of β-glucuronidase was regulated by *pecT* and *crp*. However, the cassette inserted in the opposite orientation gave an EPS$^-$ strain. When the cassette was inserted into the *Nsi*I1 site, in any orientation, the strain was EPS$^+$ and did not express β-glucuronidase. Thus, the *Bam*HI1 site is located between the transcription and the translation start sites of the EPS biosynthesis operon while the *Nsi*I site is located upstream of the promoter. The 580-bp *Nsi*I-*Bam*HI DNA fragment was sequenced (Fig. 2). A putative promoter with a –35 region containing 4 of the 6 nucleotides of the consensus (TTGTCT) at position 228 to 233 and a –10 region containing 4 of the 6 nucleotides of the consensus (TTTACT) at position 252 to 257 was identified. A putative CRP-box could be found from position 267 to 285. A JUMPstart sequence is located between nucleotides 402 and 439 (Fig. 2). JUMPstart sequences are 39 nucleotide conserved sequences identified first in the 5′ region of polysaccharide biosynthesis operons but

Table 2. Expression of a *weaP*::*uidA* fusion in various growth conditions and in various backgrounds

Additional mutation	Growth condition[a]	β-glucuronidase specific activity[b]
–	Glycerol	80
–	Glycerol (anaerobiosis)	71
–	Glycerol + 0.3M NaCl	83
–	Glycerol (low osmolarity)	266
–	Glycerol (37°C)	126
–	Glycerol + leucine	74
–	Glycerol + PGA	83
–	Glycerol + plant extract	75
–	Glycerol + glucuronate	75
–	Glycerol + galacturonate	64
–	Glycerol + galactose	75
–	Glycerol + rhamnose	140
–	Glucose (0.2%)	129
–	Glucose (1%)	156
pecT	Glycerol	238
	Glucose (1%)	391
	Glycerol (low osmolarity)	1264
kdgR	Glycerol	108
pecS	Glycerol	54
	Glucose (1%)	43
crp	Glucose (1%)	930
crp pecT	Glucose (1%)	1475
dsbA	Glucose (1%)	453

[a] The bacteria were grown in M63 medium at 30°C unless overwise stated to an OD_{600} of 1.2 to 1.5. Anaerobic cultures were realized by overlaying the culture medium with paraffin oil. Fumarate (0.25%) was added as an electron acceptor. High osmolarity was obtained by adding 0.3 M NaCl to M63 medium. Low osmolarity medium was fivefold diluted M63 medium.

[b] Results presented are the average of at least three independent assays. Standard deviation was less than 20%. Activity is expressed as nmole of *p*-nitrophenol formed per min per mg of bacterial dry weight.

Table 1. Homology between *Erwinia chrysanthemi* exopolysaccharide (EPS) synthesis proteins and proteins present in data bases

Sequence from site[a]	Homology[b]	Gene[c]	Reference
*Sal*I1	*Klebsiella pneumoniae* ORF19 (79% over 44 amino acids [aa])		Arakawa et al. 1995
*Pst*I1	*E. amylovora* AsmH (78% over 65 aa)	*wza*	Bugert and Geider 1995
*Nsi*I2	*E. amylovora* AsmA (70% over 51 aa)	*wzc*	Bugert and Geider 1995
*Bgl*II2	*Salmonella typhimurium* LT2 Rfb (46% over 64 aa)	*wbaP*	Jiang et al. 1991
*Bam*HI2	*Escherichia coli* RffG (72% over 36 aa)	*rmlB*	Marolda and Valvano 1995
*Pst*I2	*E. coli* RffH (84% over 49 aa)	*rmlA*	Marolda and Valvano 1995

[a] The sites are those shown in Figure 1.

[b] The proteins deduced from the sequence were compared with those contained in data bases, by the BLAST algorithm. The best homology is presented. The percentage of identity is indicated in parentheses.

[c] Name of the corresponding gene according to the Reeves et al. (1996) gene nomenclature.

that can be found in front of other operons (Bailey et al. 1997; Hobbs and Reeves 1994).

Pathogenicity of an EPS⁻ mutant.

When potted saintpaulia plants were inoculated with bacterial suspensions containing 3 to 5 × 10^8 CFU per ml, no significant difference was observed between the wild-type strain 3937 and the EPS⁻ mutant A3165 during the course of infection (data not shown). When the plants were infected with an inoculum containing 10 times fewer bacteria, similar symptoms were observed with both strains during the first week: about three-fourths of the plants expressed no symptoms. A difference appeared after 2 weeks (Fig. 3). A majority of the A3165-infected plants exhibited no symptom while systemic maceration appeared among plants infected with the wild-type strain. After 3 weeks, the main symptom observed with the EPS⁻ mutant was a patch of maceration whereas a majority of the plants infected with the wild-type strain showed a completely macerated, inoculated leaf. Finally, after 5 weeks, more than one-third of the plants inoculated with the EPS⁻ mutant showed only a patch of maceration that remained localized at the site of inoculation and had dried out. In conclusion, when the inoculum was low, the EPS⁻ mutant was not as efficient as the wild-type strain in initiating maceration and was unable to induce a systemic soft-rot symptom.

DISCUSSION

The high efficiency of *E. chrysanthemi* pectate lyases in macerating plant tissue has led research to focus on these enzymes and on the regulation of their synthesis, and to neglect the identification of other factors that could be important for disease development. The strategy to identify such factors has been to look for *pgi* (polygalacturonate induced) genes (Beaulieu and Van Gijsegem 1990; Hugouvieux-Cotte-Pattat and Robert-Baudouy 1989). However, the *pgi* genes characterized so far are mostly pectinase genes (Shevchik and Hugouvieux-Cotte-Pattat 1997; Shevchik et al. 1997). Another approach can be undertaken to find factors regulated by the genes controlling pectate lyases synthesis, *pecS* and *pecT*. The mucoid aspect of *pecT* mutants raised the possibility that PecT could regulate EPS synthesis. We show in this paper that most of the genes involved in EPS synthesis belong to the *pecT* regulon and that EPS production contributes to the full virulence of *E. chrysanthemi*.

The majority of the fusions isolated in genes required for EPS synthesis are regulated by PecT. These fusions are all localized in the same region of the chromosome, suggesting the presence of an EPS biosynthesis gene cluster. Sequence data show that the genes identified in this region, which present homology with genes involved in EPS synthesis of other bacteria, are transcribed in the same orientation. A JUMPstart sequence is present in the regulatory region of the first gene of this cluster. The JUMPstart sequences are present in 5′ untranslated part of EPS synthesis operon mRNAs. They contain the *ops* element, which recruits RfaH, which increases the processivity of RNA polymerase and allows transcription to occur over a long distance (Bailey et al. 1997). These data led to the speculation that *E. chrysanthemi* EPS synthesis genes are grouped in an operon larger than 10 kb. Such an organization has often been reported for EPS synthesis genes. The *E. amylovora ams* genes are grouped in a 16-kb operon (Bugert and Geider 1995) and the *K. pneumoniae* K2 *cps* gene cluster produces a transcript larger than 14 kb (Wacharotayankun et al. 1992). The genes identified by partial sequencing show homology with the genes *wza*, *wzc*, *wbaP*, *rmlA*, and *rmlB*.

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TGCATTATATGCCAAATCAGCGTGTCGCGCGGGAAATTAGCCCATCCTCTGGCTATTTAA   60
NsiI
TAAGCTCATTAATGCGTTAACAAAATTATTATATGAAAATGAAATATTTATTAAGATGTC  120
TCTGATATATATAACTGTTTGTTTTTTAATGATAAAAATATCTTTGCTCATAAGAGAAAC  180
TGCATGGAATGTTAGTATTTTTCCGAAAAAAAACACCAGGAAGATATTTGTCTTGTTATA  240
AGCAAATATTTTTTACTACTCTCCGGTGTGGCGATATTGACACATCTTTAAAAATATTTA  300
                          TGTGANNNNNNTCACA

ATTCGAAACTTTAGTGTCGTTAATGTTTTTGCAAGCCATTTTTACGAGTCAGTTATGACG  360
AGTCACAGCAATAGCTGGGCGAGTCTGAAACGAAAACGGGACAGCGAGTTGGTAGCTACG  420
                                          MASYRYAYTGGTAGCTGWR

AGCCAAGGGCGGTAGCGTACCTGATGCGATTCACCCGACAGCCGCAAGCGGCGTTTCAGA  480
AGCCARGGGCGGTAGCGTR

CCCAGCCCTCCAGGACGTTGAAACCGTTCAGCCACCGGTATTCAGGCCTTCCGCCTGTTG  540

TTTGCCGATGAGCGAAGACGGAAACGATGTCAACGGATCC                      580
                                   BamHI
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Fig. 2. Sequence of the 580-bp *Nsi*I1-*Bam*HI1 DNA fragment containing the possible promoter region of the exopolysaccharide (EPS) biosynthesis operon. Potential promoter is underlined. CRP-box is boxed. JUMPstart sequence is shaded in gray. The consensus of the CRP-box and that of the JUMPstart sequence (Hobbs and Reeves 1994) are indicated under the sequence (M, A, or C; Y, C; or T; R, G, or A; W, T, or A). This sequence will appear in the EMBL data bases under the accession number AJ009561.

wza encodes an outer membrane lipoprotein of unknown function. *wzc* encodes a protein that could have an autophosphorylating protein tyrosine kinase activity (Grangeasse et al. 1997). These two genes are always clustered with a third gene, *wzb*, that encodes a phosphatase (Reeves et al. 1996). This gene has not yet been found in *E. chrysanthemi*. *S. typhimurium wbaP* encodes an enzyme that transfers galactose-1-phosphate to undecaprenyl-phosphate. *E. chrysanthemi weaP* shows only 45% homology with *wba*. Thus, we cannot conclude that these two genes encode proteins with the same function. The genes *rmlA* and *rmlB* are involved in the synthesis of dTDP-rhamnose from glucose (Köplin et al. 1993). The sequencing of the whole cluster will be necessary to identify all the genes required for EPS synthesis in *E. chrysanthemi*.

The regulation of *E. chrysanthemi* EPS synthesis is complex. Two negative regulators have been identified: *pecT* and *crp*. While a *pecT* mutation increases EPS production, a mutant overproducing PecT synthesizes less EPS than the wild-type strain (Castillo and Reverchon 1997), suggesting a direct role of PecT in repression. While *pecT* links EPS production with that of pectate lyases, *crp* has opposite effects on EPS and pectate lyases synthesis since a *crp* mutant does not synthesize any pectate lyase (Reverchon et al. 1997). Identification of a potential CRP-binding box downstream of the *wza* putative transcription start could explain the repressor role of *crp:* binding of CRP at this position would inhibit fixation of the RNA polymerase (Kolb et al. 1993). The increased EPS synthesis in a low osmolarity medium may help the bacteria to retain salts and nutrients in this less favorable growth condition, which could be encountered by the bacteria in the soil or in plant tissue.

Infection of *Saintpaulia ionantha* with the wild-type strain 3937 never gives 100% of systemic maceration but a part of the infection stops at the stage of one macerated leaf. *E. chrysanthemi* EPS$^-$ mutants have a reduced virulence on saintpaulia. With a low inoculum, not only was systemic maceration never observed but also, after 5 weeks, some plants showed no symptoms or a dried-out maceration patch at the site of inoculation. Appearance of the symptoms seems delayed. Two hypotheses can be proposed on the role of EPS in the infection process: (i) EPS itself could be a virulence factor, like stewartan and amylovoran, blocking xylem vessels;

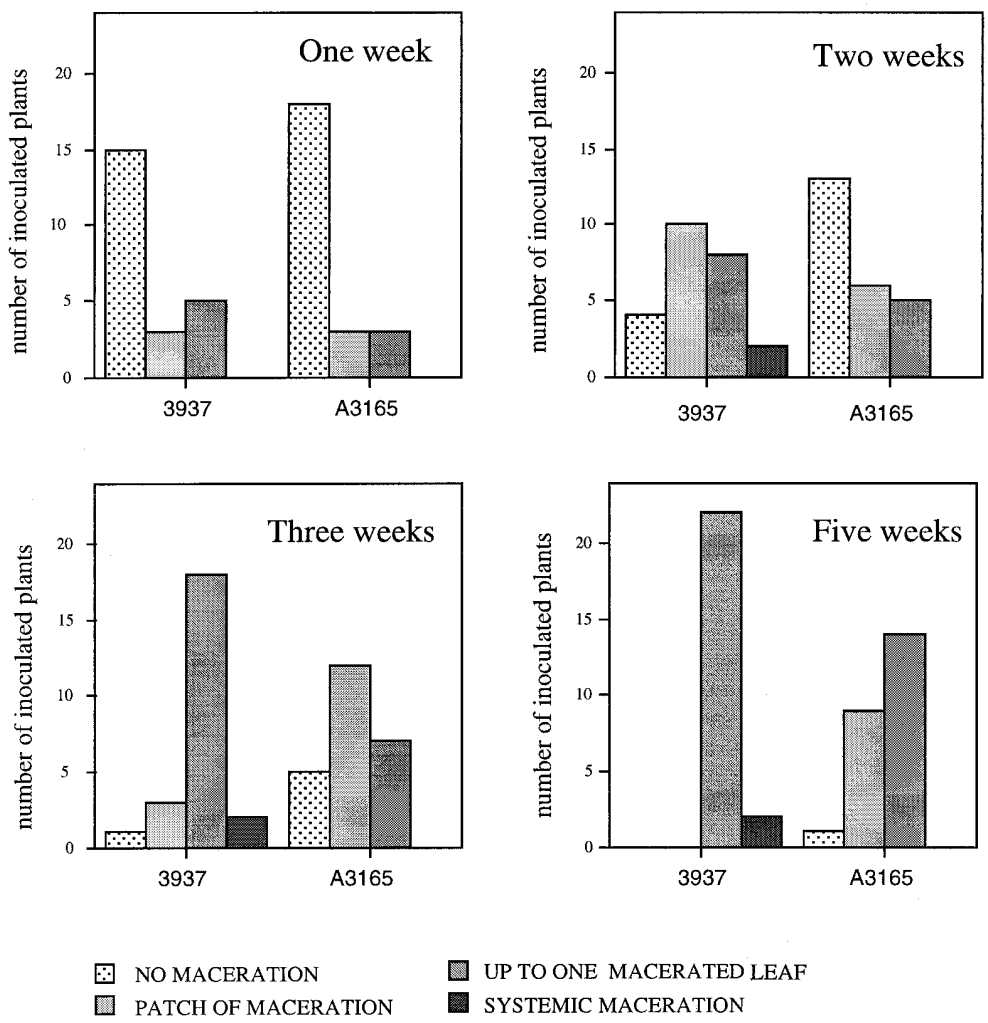

Fig. 3. Distribution of responses given by the EPS$^-$ mutant strain A3165 and the wild-type strain 3937 on African violets. Each strain was inoculated to 24 plants as described in the Materials and Methods.

(ii) production of EPS could protect *E. chrysanthemi* and help it to survive in the soil or in the plant during the first steps of infection by creating a favorable environment. The delay before appearance of the first symptoms could correspond to the time required for the mutants to multiply in the less protected in planta condition due to the absence of EPS. The plant defense mechanisms could more easily kill the bacteria during this lag time. While amylovoran is produced by all the strains of *E. amylovora*, EPS produced by various *E. chrysanthemi* strains have a different composition (Yang et al. 1994, 1996), suggesting that the type of molecule produced is not of importance for this bacteria. Thus, the role of EPS may be to protect *E. chrysanthemi* cells in the plant. However, the coordinate synthesis of EPS and other virulence factors and the reduced virulence of EPS$^-$ mutants suggest that EPS is an authentic virulence factor in *E. chrysanthemi*.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions.

Bacterial strains and plasmids used in this study are described in Table 3. *E. chrysanthemi* and *E. coli* cells were grown at 30 and 37°C, respectively, in Luria broth medium or M63 minimal medium (Miller 1972) supplemented with a carbon source (0.2%; except PGA [polygalacturonate], 0.4%) and, when required, with amino acids (40 µg/ml) and the following antibiotics at the concentrations indicated: ampicillin, 100 µg/ml; kanamycin, 50 µg/ml; chloramphenicol, 20 µg/ml; and tetracycline, 20 µg/ml. M63 diluted fivefold was used as a low osmolarity medium. Production of EPS by *E. chrysanthemi* strains was tested on 1% glucose-containing minimal medium plates.

Genetics techniques.

Transduction with phage ϕEC2 was done as described (Resibois et al. 1984). Gene mapping on the chromosome was performed by conjugation with pULB110 as previously reported (Hugouvieux-Cotte-Pattat and Robert-Baudouy 1989). Mutagenesis with transposon Tn*5-B21* was performed by mixing 0.2 ml of an overnight culture of strain S17-1/pSUP102-Tn*5-B21* with 0.2 ml of an overnight culture of the *E. chrysanthemi* recipient cell on an M63 agar plate. After 5 h at 30°C, cells resuspended in 1 ml of M63 medium and dilutions were spread on glucose-plus-tetracycline plates. Marker exchange recombinations were obtained after growth in a low-phosphate-concentration medium as described (Roeder and Collmer 1985).

Enzyme assays.

β-Glucuronidase assays were performed with toluenized cells grown to exponential phase with *p*-nitrophenyl-β-D-glucuronate as the substrate (Novel et al. 1974). β-Galactosidase assays were performed with toluenized cells grown to exponential phase with *o*-nitrophenyl-β-D-galactose as the substrate (Miller 1972). Pectate lyase activity was assayed on culture supernatant as described (Moran and Starr 1969).

Recombinant DNA techniques.

E. chrysanthemi chromosomal DNA was prepared according to Ausubel et al. (1987). Preparations of plasmid DNA, restriction digestions, ligations, DNA electrophoresis, transformations, and electroporations were carried out as described by Sambrook et al. (1989). Nucleotide sequence analysis was performed by the chain termination method with double-stranded DNA templates, with the Pharmacia T7 sequencing kit (Pharmacia, Uppsala, Sweden). The *uidA*-kan cassette was extracted from plasmid pUIDK3 (Bardonnet and Blanco 1992).

Pathogenicity tests.

The plant assay was carried out on potted *Saintpaulia ionantha* cv. Blue rhapsody (African violets) as previously described by Expert and Toussaint (1985) except that each

Table 3. Bacterial strains, plasmids, and phage used in this study

Designation	Description	Reference or source
Strains		
Erwinia chrysanthemi		
3937c	Wild type	Laboratory collection
A350	*lmrT*^{c} *lacZ*	Hugouvieux-Cotte-Pattat and Robert-Baudouy 1985
A837	*lmrT*^{c} *lacZ kdgR*	Condemine and Robert-Baudouy 1987a
A1524	*lmrT*^{c} *lacZ pecS*::MudIIPR13	Reverchon et al. 1994
A2174	*lmrT*^{c} *lacZ pecT*::Cm	Surgey et al. 1996
A2507	*lmrT*^{c} *lacZ crp*::Cm	Reverchon et al. 1997
A2575	*lmrT*^{c} *lacZ eps*::Tn*5-B21*	This work
A2628	*lmrT*^{c} *lacZ dsbA*::Cm	Laboratory collection
A2952	*lmrT*^{c} *lacZ weaP*:: *uidA*-kan	This work
A3165	*weaP*:: *uidA*-kan	This work
Escherichia coli		
NM522	Δ(*lac-proAB*) *thi hsd-5 supE* (F′ *proAB*$^+$ *lacIq lacZ* ΔM15)	Stratagene, La Jolla, CA
S17-1	*hsdR pro* RP4-2 Tc::Mu Km::Tn*7*	Simon et al. 1983
Plasmids		
pBS	Ap^r *lacZ*′	Stratagene
pB21	pSUP102::Tn*5-B21*	Simon et al. 1989
pUIDK3	Contains a *uidA*-kan cassette	Bardonnet and Blanco 1992
Phage		
ϕEC2	*E. chrysanthemi* generalized transducing phage	Resibois et al. 1984

bacterial strain was tested on 24 plants and prepared as follows: Bacteria were cultivated in M63 minimal medium overnight from a fresh L-agar plate and diluted in M63 medium at a concentration of 3 to 5×10^8 or 3 to 5×10^7 CFU per ml and 100 µl was inoculated into a leaf wounded with a scalpel. Plants were maintained in a growth chamber at 27 to 30°C under high moisture conditions, with 16 h of illumination daily.

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