Xanthomonas campestris Contains a Cluster of hrp Genes Related to the Larger hrp Cluster of Pseudomonas solanacearum

Matthieu Arlat¹, Clare L. Gough², Christine E. Barber¹, Christian Boucher², and Michael J. Daniels¹

¹The Sainsbury Laboratory, Norwich Research Park, Colney, Norwich NR4 7UH, United Kingdom. ²Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, INRA-CNRS, 31326 Castanet-Tolosan Cedex, France.

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All Xanthomonas campestris pathovars tested contain DNA which hybridizes to the large hrp gene cluster of Pseudomonas solanacearum (C. A. Boucher, F. Van Gijsegem, P. A. Barberis, M. Arlat, and C. Zischke, J. Bacteriol. 169:5626-5632, 1987). Clones carrying these sequences were isolated from genomic libraries of X. campestris pv. campestris and vitians. Mutagenesis of the corresponding genomic regions of both pathovars gave strains defective in both pathogenicity and hypersensitive response induction. X. c. pv. campestris contained a hrp gene cluster covering about 25 kb, which was homologous and colinear over a continuous 19-kb DNA region with the P. solanacearum hrp cluster. Cross-complementation showed that X. c. pv. vitians and X. c. pv. campestris hrp sequences are functionally interchangeable, but the source of the hrp genes did not determine the compatibility-incompatibility of the host-pathogen interaction. One X. c. pv. campestris Hrp+ mutant was "complemented" by specific subclones of the P. solanacearum hrp cluster, suggesting the existence of some functional homology between the clusters of the two species. Expression of hrp genes (studied by lacZ fusions) was repressed in rich medium, and in minimal medium the level of expression depended on the carbon source supplied to the cells. Transcription of hrp genes was not regulated by genes that control the synthesis of extracellular enzymes, which are required for pathogenicity. In addition X. campestris Hrp+ mutants produced wild-type levels of these extracellular enzyme activities. These results suggest the existence of two independent sets of pathogenicity genes that are regulated differently.

Additional keywords: lettuce, pepper, turnip.

The hypersensitive response (HR) of higher plants is a defense reaction characterized by the rapid, localized necrosis of plant tissues, which is specifically induced by pathogenic microorganisms (Klement 1982). This reaction occurs in so-called incompatible interactions involving pathogens and either nonhost or resistant plants. It has been suggested that factors of the pathogen inducing HR in nonhosts might also contribute to pathogenicity in susceptible hosts (Klement 1982). This hypothesis has been confirmed for several phytopathogenic bacteria. Molecular genetic studies have revealed a class of pathogenicity genes, designated hrp genes, which are essential both for the development of disease symptoms on susceptible plants and for the elicitation of HR on nonhost or resistant plants in Pseudomonas syringae van Hall (Anderson and Mills 1985; Lindgren et al. 1986; Huang et al. 1988), Pseudomonas solanacearum (Smith) Smith (Boucher et al., 1987, Huang et al. 1990), Erwinia amylovora (Burrill) Winslow et al. (Beer et al. 1991; Barny et al. 1990), and Xanthomonas campestris (Pammel) Dowson (Bonas et al. 1991; Kamoun and Kado 1990). The apparent dual role of hrp genes suggests that plants have developed resistance mechanisms based on recognition of bacterial determinants that control pathogenicity. The identification of these determinants and of the biochemical functions encoded by hrp genes could give insight into one of the basic defense mechanisms of plants.

Most of the hrp genes are organized in a large cluster in all the bacteria studied so far. The hrp clusters are conserved among P. solanacearum strains (Boucher et al. 1988) and among P. syringae pathovars (Lindgren et al. 1988), but no homology was found between the hrp clusters of these two species (Boucher et al. 1987). Recently Beer et al. (1991) have reported the existence of homology between the hrp clusters of E. amylovora and P. syringae, suggesting that these two species share some core pathogenicity functions. Similarly in our previous work, we established that the large hrp cluster of P. solanacearum that covers about 23 kb possesses homologous sequences in the genome of all pathovars tested of X. campestris (Boucher et al. 1987).

In this paper, we report the cloning of these homologous DNA sequences from two X. campestris pathovars, X. c. pv. campestris (Pammel) Dowson and X. c. pv. vitians (Brown) Dye, the causal agents of black rot of crucifers and leaf spot of lettuce, respectively, and we show that they carry related hrp genes. In X. c. pv. campestris these hrp genes form a cluster of approximately 25 kb. This hrp cluster is homologous and colinear over 19 kb with the hrp cluster of P. solanacearum. Functional complementation was detected between some of the hrp sequences of the two pathogens. We have also established that the loss of pathogenicity of X. campestris Hrp+ mutants is not due to a failure to produce extracellular enzymes known to be required for pathogenicity. Finally, data concerning the expression of X. c. pv. campestris hrp genes ex planta are given.

Address correspondence to M. J. Daniels, The Sainsbury Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UH, UK.

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MATERIALS AND METHODS

**Bacteria, plasmids, phage, and transposon.** The bacterial strains, plasmids, phage, and transposon used in this work are listed in Table 1.

**Growth media and conditions and genetic techniques.** Growth media, conditions of incubation, and antibiotic selection have been described previously (Collinge et al. 1987; Daniels et al. 1984a; Turner et al. 1984, 1985). Plasmid transfer and other genetic techniques were described by Turner et al. (1984). Marker exchange followed the procedure of Turner et al. (1985), except that for *X. c. pv. vitians* the plasmid used to displace the plAFR3 derivative, plJ3228 carrying the Ω cassette, was plJ3011, a spontaneous spectinomycin susceptible (Sp') derivative of pPH11 (Sawczyn et al. 1989). Marker exchange was verified by tetracycline (Te) sensitivity and by Southern analysis (Turner et al. 1985).

**DNA manipulation.** Plasmid purification, restriction endonuclease mapping, gel electrophoresis, and DNA ligation were performed by standard procedures (Maniatis et al. 1982). DNA restriction fragments were isolated from agarose gels using a Geneclean Kit (Bio 101, La Jolla, CA).

Southern transfers and colony lifts were performed with Biodyne (Pall, Glen Cove, NY) membranes as recommended by the manufacturer. High-stringency hybridization was performed at 42°C in 5X SSC (1X SSC = 0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0)-50% formamide, followed by three washes in 2X SSC-0.1% sodium dodecyl sulfate (SDS) at room temperature and two washes in 0.1X SSC-0.1% SDS at 50°C. Low-stringency hybridization was performed at 37°C in 5X SSC-50% formamide, followed by three washes in 2X SSC-0.1% SDS at room temperature.

**Table 1. Bacteria, plasmids, phage and transposon used in this study**

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<tr>
<th>Designation</th>
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<td>S17-1</td>
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<td>polA1 rha his Naïl' Rif'</td>
<td>Maniatis et al. 1982</td>
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<td>C600</td>
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<td>9001</td>
<td>Rif'</td>
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<td>9001-Δ</td>
<td>9001-Δ, delta htp, Sp', Sm', contains plJ3011</td>
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<tr>
<td>8004</td>
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<td>8258</td>
<td>Sm', Cm', contains pPH11J</td>
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<td>8237</td>
<td>rfp derivative of 8004, protease-minus, polygalacturonate lyase-minus, endoglucanase-minus amylose-minus; non-pathogenic</td>
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<td>8288</td>
<td>xps derivative of 8004, unable to secrete protease, endoglucanase, polygalacturonate lyase, amylose; non-pathogenic</td>
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<td>516-9</td>
<td>8004::Tn5, protease-minus</td>
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<td>8004::Tn5, endoglucanase-minus</td>
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<td><strong>Plasmids</strong></td>
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<td>b221 (att', int'), red−, Oam, pam, cl857</td>
<td>N. Kleckner</td>
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<td><strong>Transposon</strong></td>
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<td>Tn5 carrying a promotorless lacZ gene cloned into IS50L</td>
<td>Simon et al. 1989</td>
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*Abbreviations: Cm, chloramphenicol; Gm, gentamycin; Km, kanamycin; NaI, nalidixic acid; Rif, rifampicin; Sm, streptomycin; Sp, spectinomycin; Te, tetracycline.*
*E. coli* C2110 Nal’Rif” (nalidixic acid resistant, rifampicin resistant). Selection on L-NalKmTc gave colonies carrying pIJ3225::Tn5-B20.

**Pathogenicity tests.** Pathogenicity of *X. c. pv. campestris* strains was tested on turnip (*Brassica campestris* ‘Just Right’) by inoculation of leaf margins of mature turnip leaves as described by Gough et al. (1988), by infiltration of mature leaves, or by needle-inoculation of seedlings as described by Daniels et al. (1984a). In this latter test, disease progression was assessed as described by Osbourn et al. (1990). Mutants that rotted more than 80% of the seedlings during the test were considered pathogenic; mutants that rotted less than 20% of the inoculated seedlings and that did not induce any visible symptoms on at least 75% of the remaining seedlings after 7 days of incubation were considered nonpathogenic. Mutants that induced intermediate phenotypes on seedlings were considered attenuated.

Pathogenicity of *X. c. pv. vitians* derivatives was tested on lettuce plants (varieties Buttercrunch and Winter Density). Suspensions of bacteria at 10^7 and 10^8 cells ml^{-1} were infiltrated into the lamina of fully expanded leaves of 3- to 6-wk-old plants. After inoculation, plants were kept at 28°C in sealed plastic bags to maintain high humidity for 4–5 days. Under these conditions the pathogenic strain 9001 induced blackening and rotting of infiltrated tissues.

**HR tests.** HR-inducing ability of *X. c. pv. campestris* strains was tested on pepper cultivar Early Calwonder-10R (ECW-10R) (Minsavage et al. 1990). Suspensions of bacteria at 10^7 or 10^8 cells ml^{-1} in water were infiltrated into the intercellular spaces of mature leaves. The wild type strain induced yellow-green confluent necrosis that appeared within 24 hr and did not spread beyond the inoculated area.

HR-inducing ability of *X. c. pv. vitians* strains was tested by infiltrating turnip leaves (cv Just Right) as previously described (Conrads-Strauch et al. 1990).

**β-galactosidase activity assay.** Screening of the different Tn5-B20 fusions for expression was performed in rich medium (NYGB, Turner et al. 1984) and in MME minimal medium (K_2HPO_4, 10.5 g L^{-1}; KH_2PO_4, 4.5 g L^{-1}; (NH_4)_2SO_4, 1 g L^{-1}; Difco Casamino acids, 0.15 g L^{-1}; and 1 mM MgSO_4) supplemented with sucrose (20 mM). *X. c. pv. campestris* mutants containing Tn5-B20 insertions were grown to midexponential phase in NYGB at 28°C, washed twice with sterile water, and resuspended at 3 × 10^8 cfu ml^{-1} in MME + sucrose or NYGB. Cultures were shaken at 28°C for 14 hr and then assayed for β-galactosidase activity as described below. For NYGB-grown cells, the culture was also assayed for β-galactosidase activity at 8 × 10^8 cfu ml^{-1}.

Assays to test the activity of different carbon sources and nutrients on the expression of A2, B2, or F8 fusions were performed in MME containing supplements as in Figure 4, and used at 20 mM except for the following: glycerol, 40 mM, pyruvate, 40 mM, Peptone (Oxoid, 10 g L^{-1}), Casamino acids (Difco, 5 g L^{-1}), yeast extract (Beta-
β-galactosidase assays were carried out as described by Miller (1972) following the modifications of Boivin et al. (1990).

Detection of extracellular enzyme activity in cell-free liquid culture medium. Relative activities of endoglucanase, amylase, and protease were measured by diffusion assays into substrate-containing agar plates (Dow et al. 1987; Tang et al. 1987). Polygalacturonate lyase (PGL) was assayed spectrophotometrically using bacteria that had been grown with polygalacturonate to induce the enzyme (Dow et al. 1987).

RESULTS

Cloning and physical study of X. c. pv. campestris and X. c. pv. vitians DNA sequences hybridizing with the hrp gene cluster of P. solanacearum. A pLAFLR3 library of X. c. pv. vitians 9001 DNA (Roberts et al. 1987) was screened under low stringency conditions with subcloned DNA fragments from cosmids pVir2, which carries a large portion of the hrp cluster of P. solanacearum strain GM1000 (Fig. 1; Boucher et al. 1987). Two cosmids, pLJ3220 and pLJ3221, carrying overlapping DNA fragments that showed strong hybridization under low stringency conditions with parts of pVir2 (Fig. 1) were identified. The orientation of X. c. pv. vitians sequences presented in Figure 1 was deduced as described below.

Subcloned DNA fragments from pLJ3220 were in turn used to screen a previously constructed pLAFLR1 library of X. c. pv. campestris 8004 DNA (Daniels et al. 1984b). Six cosmids with overlapping inserts were identified (Fig. 1). The relative orientation of the inserts shown in Figure 1 was deduced from experiments presented below. Hybridization experiments using these six cosmids as probes against EcoRI or BamHI digested genomic DNA of strain 8004 showed that the inserts were colinear with genomic DNA (data not shown).

The homology between the hrp sequences of P. solanacearum and the corresponding region of X. c. pv. campestris is summarized in Figure 1. Hybridization under low stringency conditions of EcoRI or BamHI digests of these six cosmids with pVir2 as probe defined the restriction fragments of these cosmids carrying DNA sequences homologous to pVir2. The X. c. pv. campestris fragments homologous to the pVir2 probe formed a continuous region of about 25 kb that corresponded to the pLJ3225 insert (Fig. 1). The same Southern transfers were rehybridized under low stringency conditions with a probe corresponding to pAFE8, which overlaps pVir2 and carries the leftmost hrp sequences and the adjacent genomic sequences of P. solanacearum (Arlat et al. 1990) (Fig. 1). We found that pLJ3226 and pLJ3227 did not hybridize with this latter probe and in the other cosmids only one EcoRI fragment, common to all the clones, was detectable. This unique EcoRI fragment is the leftmost EcoRI fragment carried by pLJ3225 (Fig. 1). These hybridization results allowed us to orient the X. c. pv. campestris genomic region defined by the six cosmids relative to the P. solanacearum region defined by pVir2 and pAFE8 (Fig. 1). They also suggested that the P. solanacearum sequences adjacent to the left border of the hrp cluster do not have homologues in the X. c. pv. campestris region studied (Fig. 1). To confirm this, we hybridized EcoRI and EcoRI–BamHI digests of pVir2 and pAFE8 with each of the six X. c. pv. campestris clones. All the EcoRI restriction fragments of pVir2 carried DNA sequences homologous to the six X. c. pv. campestris clones (Fig. 1). However only the right part of pAFE8, which carries hrp sequences, gave hybridization (Fig. 1). The pattern of hybridization obtained with each probe also confirmed the orientation of the two genomic regions previously deduced.

pVir2 and pAFE8 (P. solanacearum) and pLJ3225 and pLJ3222 (X. c. pv. campestris) were subcloned (data not shown) and the subclones were digested with different restriction enzymes to generate a set of subfragments that were isolated after agarose gel electrophoresis. Twelve independent subfragments ranging from 1.5 to 7.5 kb covering the genomic region defined by pLJ3225 and pLJ3222 (Fig. 2) and 21 different subfragments ranging from 0.5 to 7.5 kb covering the genomic region defined by pVir2 and pAFE8 were isolated (Fig. 2). The latter subfragments were electrophoresed, transferred to membranes, and hybridized under high stringency conditions with probes prepared from each of the 12 X. c. pv. campestris subfragments (Fig. 2). A region of pLJ3225 encompassing about 19 kb hybridized in a colinear manner with a region of about 18 kb of the hrp cluster of P. solanacearum. The data also confirmed the relative orientation of the two regions. The left and central parts of the inserts carried by pVir2 and pLJ3225 gave hybridization but the right parts did not. It is however interesting to note that the previous low stringency hybridization experiments suggested relatedness between pLJ3225 and the rightmost EcoRI fragments of pVir2 (Fig. 1). Either weak overall homology exists between these two regions or the homology extends only over a very small stretch of DNA. Finally, as expected, no hybridization was detected between subfragments specific to pLJ3222 and pAFE8.

Hybridization between the two X. c. pv. vitians clones and the six X. c. pv. campestris clones allowed us to orientate and place the X. c. pv. vitians DNA relative to the corresponding regions of X. c. pv. campestris and P. solanacearum (Fig. 1).

Mutagenesis of X. c. pv. vitians DNA sequences: Hrp phenotype. pLJ3220 was used to generate a localized insertion-deletion mutant of X. c. pv. vitians. The cosmid was first digested with BamHI and the internal fragments were discarded and replaced with the Δ fragment (Prentki and Krisch 1984) previously restricted with BamHI (Fig. 1). The Δ-containing cosmid, designated pLJ3228 was transferred into the wild-type strain 9001 of X. c. pv. vitians. The insertion-deletion mutation was then marker-exchanged into the X. c. pv. vitians genome.

The insertion-deletion mutant, designated 9010, was unable to induce disease symptoms on lettuce or to elicit HR in turnip leaves. The Hrp phenotype of mutant 9010 showed that the DNA sequences of X. c. pv. vitians deleted or interrupted by the Δ fragment insertion encode hrp functions.

Tn5-B20 mutagenesis of X. c. pv. campestris DNA sequences. pLJ3225 was mutagenized in E. coli with Tn5-
B20, which contains a lacZ reporter gene. Thirty-three insertions distributed through the insert were obtained.

Each Tn5-B20 insertion was marker-exchanged into *X. c. pv. campestris* 8004. The position and orientation of these marker-exchange insertions are shown in Figure 3.

All mutants were tested for pathogenicity on turnip seedlings and mature leaves, and HR-inducing ability was tested in pepper leaves. The results of these experiments are summarized in Figure 3 and show that pIJ3225 carries a region of approximately 25 kb that is required both for pathogenicity and HR-inducing ability. The Tn5-B20 insertions that produce an altered phenotype on plants are divided into three groups separated by two regions of about 2 and 4 kb, which do not appear to control either pathogenicity on turnip or HR-inducing ability on pepper. The leftmost group was defined as a Hrp^- group, because these mutants induced delayed and partial HR on pepper plants and were attenuated on turnip seedlings, rather than nonpathogenic like the Hrp^- mutants in the other groups.

**Cross-complementation experiments between *X. c. pv. vitians* and *X. c. pv. campestris* hrp sequences.** pIJ3225 carrying *X. c. pv. campestris* DNA was introduced into the *X. c. pv. vitians* mutant 9010. The resulting strain produced disease symptoms on lettuce and induced a partial and delayed HR on turnip.

Similarly pIJ3221 carrying *X. c. pv. vitians* DNA was introduced into Hrp^- mutants of *X. c. pv. campestris*, carrying Tn5-B20 insertions G2, A2, B6, B3, A3, and B2 (hereafter, the Hrp^- mutants of *X. c. pv. campestris* will be designated by the letters Xch followed by the number of the insertion they carry, i.e., XchG2, XchA2, XchB6, XchB3, XchA3, and XchB2). The cosmid restored the ability to induce HR on pepper to mutants XchB6, XchB3, and XchA3, but not to mutants XchG2, XchA2, and XchB2. These results were expected as pIJ3221 carries DNA sequences homologous to the sequences altered by insertions B6, B3, and A3, but does not contain sequences homologous to the sequences mutated by insertions G2 and A2. For mutant XchB2, our hybridization data are not accurate enough to determine whether pIJ3221 carries DNA sequences homologous to the sequences altered by insertion B2, and therefore we cannot determine whether the inability to complement this mutation is due to the absence of homologous sequences in pIJ3221 or to other reasons.

**Cross-complementation between *X. c. pv. campestris* and *P. solanacearum* hrp clusters.** pVir2 was introduced into the same set of *X. c. pv. campestris* Hrp^- mutants. For each mutant three transconjugant colonies were picked and infiltrated into pepper leaves. None of the transconjugants obtained with mutants XchA2, XchB6, XchB3, XchA3, and XchB2 were able to induce any reaction on pepper. However, one of the transconjugants from XchG2 induced a partial and delayed HR. The plasmid harboured by the HR-inducing transconjugant, designated pVir2-1, carried a spontaneous deletion (Fig. 1). This deletion removes the central part of the pVir2 insert but does not affect the DNA sequences homologous to the region surrounding the G2 insertion. Two other XchG2 transconjugants that remained HR-negative harboured pVir2, which was apparently unchanged.

Two derivatives of pVir2, pGM1731, and pGM1737, (which carry inserts from the left part of the hrp cluster [Fig. 1], including the region homologous to the G2 region)

![Fig. 2. Structural homology between the hrp region of *Pseudomonas solanacearum* and the related region of *Xanthomonas campestris* pv. *campestris*. The dotted lines above the *X. c. pv. campestris* restriction map represent the subfragments of this region used as probes in hybridization experiments against subfragments of the *P. solanacearum* region, which are shown by the bars below the *P. solanacearum* map. The shaded zones show the *P. solanacearum* subfragments that were detected with each *X. c. pv. campestris* probe under high stringency conditions. The hatched bars represent the hrp gene clusters of *P. solanacearum* and *X. c. pv. campestris* (see Fig. 3). Abbreviations: E, EcoRI; B, BamHI.](image)
were also introduced into XchG2. Three transconjugants colonies containing each plasmid were isolated and tested in pepper leaves. The three pGMI731-containing colonies elicited a delayed and partial HR, whereas the transconjugants harbouring pGMI737 did not induce any reaction. Mutants XchA2, XchB6, and XchA3 carrying pGMI731 and pGMI737 were unable to induce any reaction in pepper leaves.

XchG2 carrying pGMI731, pGMI737, pVir, or pVir2, or pVir2-1 was cultured nonselectively in MME supplemented with sucrose, and the loss of the Tc resistance marker carried by the plasmids was measured. The larger plasmids pVir2 and pGMI737 were slightly less stable than their shorter counterparts pVir2-1 and pGMI731 (data not shown). However, the difference in stability did not seem to be enough to explain the differences in complementing ability.

Expression of *X. c. pv. campestris hrp* genes. *X. c. pv. campestris* 8004 is devoid of β-galactosidase activity (Tang et al. 1991); transcriptional fusions to lacZ induced by Tn5-B20 insertions were therefore used to monitor the expression of *hrp* genes.

After growth of each mutant in MME + sucrose, about 50% of the strains did not show a significant level of β-galactosidase (<10 Miller units, Miller 1972) (Fig. 3), presumably because of insertion in the wrong orientation or in a nontranscribed region. For the other strains, the level varied from 100 to 750 units, probably reflecting the activity of a transcription unit.

Mutants XchA2 and XchB2 (Lac + in MME + sucrose), and XchF8 (Lac -) were used to test the effect of various carbon sources and nutrient supplements on the expression of *hrp* genes. No significant activity was detected with XchF8, whatever carbon source was used. However, the β-galactosidase activity varied 37.5-fold for mutant XchA2 (Fig. 4) and 18-fold for XchB2 (data not shown), according to the carbon source. Generally the activity for XchA2 was higher than that for XchB2. The relative effect of each carbon source was very similar for both mutants. There was no obvious correlation between the β-galactosidase activity induced and the ability of the carbon sources to support vigorous growth of the bacteria (data not shown).

β-galactosidase activity was also measured after growth in minimal medium containing sucrose in addition to each of the 18 carbon sources or supplements (Fig. 4). Peptone, yeast extract, and casamino acids strongly repressed the expression of the *hrp* genes. With the exception of glutamate, glycerol, and fructose, all the substances had significant repressive properties.

For each mutant producing a significant activity in MME (Fig. 3), β-galactosidase activity was measured following growth in rich medium. No significant activity (<10 units) was detected during exponential growth. However all the mutants produced significant activity (20–70 units) when they reached stationary phase, although the level was in all cases at least sevenfold lower than that measured in MME + sucrose (data not shown).

**Hrp functions and production of extracellular enzymes.** *X. c. pv. campestris* 8004 produces polysaccharide (EPS) and a range of extracellular enzymes including protease (Ptr), polygalacturonate lyase (Pgl), endoglucanase (Egl) and amylase (Aml) activities. Prt and EPS are important for pathogenicity (Dow et al. 1990; Tang 1989; Tang et al. 1987).

All the *X. c. pv. campestris* Hrp - or Hrp +/− mutants produced wild-type levels of each extracellular enzyme after growth on NYGB or MME + sucrose, and colony appearance indicated normal EPS production. Similarly the *X. c. pv. vitians* Hrp − mutant produced wild-type levels of Prt and Egl (the parent strain does not produce Pgl or Aml). Previously isolated Prt - or Egl - mutants of *X. c. pv. campestris* (516-9 and 8409 respectively) as well as mutant 8288 unable to secrete extracellular enzymes elicited on pepper a HR identical with that induced by the wild-type strain.

The *X. c. pv. campestris rpf* mutant 8237 (Tang et al. 1991) and strain 8004 (pLJ3079)(Tang et al. 1990) are unable to induce disease and produce lower amounts of extracellular enzymes and EPS. However both strains induced

![Fig. 3. Locations and orientations of Tn5-B20 insertions in pLJ3225 insert. The vertical bars show the position of insertions marker-exchanged into the *Xanthomonas campestris* pv. *campestris* genome. Circles show insertions that caused an Hrp - phenotype. Squares represent insertions that gave a Hrp + phenotype, whereas triangles show insertions that did not affect the phenotype on plants. Filled symbols represent insertions that produced significant β-galactosidase activity in MME + sucrose, whereas open symbols correspond to silent insertions. The arrows indicate the transcription orientation of the lacZ gene. Abbreviation: E, EcoRI; B, BamHI.](image-url)
normal HR in pepper leaves. pIJ3225 carrying insertions A2 or B2 was introduced into both the wild-type strain 8004 and into the rpf mutant 8237. No significant difference in β-galactosidase activity ascribable to the rpf mutation was observed after growth of the cells in NYGB or in MME + sucrose, glucose, or pyruvate (data not shown). Also, introduction of pIJ3079 into mutants XchA2 and XchB2 did not affect β-galactosidase production after growth of the cells in the same media.

DISCUSSION

We have used *P. solanacearum* hrp genes to clone homologous sequences of *X. c. pv. campestris* and *X. c. pv. vitians* and shown by mutation that the latter carry hrp genes. In *X. c. pv. campestris* the hrp genes form a cluster covering approximately 25 kb, which is organized into three regions controlling HR-inducing ability and pathogenicity, separated by small domains wherein mutations have a Hrp^+ phenotype. The relatedness to the genes of *P. solanacearum* extends over 19 kb. There was no homology between the sequences flanking the hrp clusters of the two species, which are not considered to be closely related (De Vos et al. 1985). It is possible that the hrp clusters of both pathogens have evolved separately from a common origin and that the selective pressure was higher for the hrp sequences than for the surrounding sequences, or alternatively horizontal transmission may have taken place more recently. Restriction fragment length polymorphism studies on 52 different strains of *P. solanacearum* suggested that the hrp sequences have coevolved with the rest of the genome (Boucher et al. 1988). It will be interesting to undertake similar studies for *X. campestris* pathovars.

The *P. solanacearum* hrp cluster is located on a megaplasmid (Boucher et al. 1986). Attempts to detect megaplasmids or other indigenous plasmids in *X. c. pv. campestris* 8004 have failed, and we therefore assume that the hrp cluster is located on the chromosome.

The hrp sequences of *X. c. pv. campestris* and *X. c. pv. vitians* cross-hybridize, and each can complement mutations in the other for the induction of disease and elicitation of HR, suggesting that these two hrp regions are highly conserved. It is noteworthy that the compatibility or incompatibility of a particular pathovar-plant interaction is not determined by the source of the hrp genes used to complement the mutation. Thus other factors (perhaps avirulence genes) must determine the choice between disease and HR. It is likely that all pathovars of *X. campestris* carry a hrp cluster analogous to that of *X. c. pv. campestris*, and studies are under way to determine if the clusters we have identified are related to the hrp cluster of *X. c. pv. vesicatoria* isolated by Bonas et al. (1991) or to the hrp locus identified by Kamoun and Kado (1990).

One *X. c. pv. campestris* mutant, XchG2, could be partially complemented by certain subclones carrying part of the *P. solanacearum* hrp region, whereas other subclones or the whole region could not complement the mutation. The explanation of this is not known.

Transcription of *X. c. pv. campestris* hrp genes is regulated in response to the nutritional status of the bacterium, being repressed in rich medium like hrp genes of *P. solanacearum* (Arlat et al. 1990), *P. syringae* pv. *glycinea* (Huynh et al. 1989), *P. s. phaseolicola* (Rahme et al. 1991) and *E. amylovora* (Beer et al. 1991). Expression of the hrp genes depended on the carbon source supplied. Sucrose, glutamate, and glycerol gave the highest levels of expression, followed by tricarboxylic acid (TCA) cycle intermediates, whereas casamino acids, yeast extract, and peptone strongly repressed transcription. It is interesting to compare our data with the results obtained by Huynh et al. (1989) on transcription of the *P. s. glycinea* avrB gene, which is itself controlled by hrp genes. The general pattern of nutritional regulation of avrB expression is similar to that of *X. c. pv. campestris* hrp genes. There are some differences in the relative effects of different carbon sources that may reflect differences in metabolic pathways in the two bacteria. In *Pseudomonas* species synthesis of carbohydrate utilization enzymes and transport systems is subject to catalytic repression by TCA cycle intermediates (Lessie and Phibbs, 1984) but this occurs only to a slight extent in *X. c. pv. campestris* (Whitfield et al. 1982). In *Pseudomonas aeruginosa* glycerol derepresses TCA cycle enzymes (Hamlin et al. 1967), whereas it has the opposite effect in *X. c. pv. campestris* (Whitfield et al. 1982). Huynh et al. (1989) suggested that sucrose, which is abundant in leaf tissues, contributes to induction of avrB and hrp genes during infection. It is likely that the regulation of these classes of pathogenicity gene helps the bacterium to exploit its host more efficiently.

hrp genes are not involved in regulation of production or export of extracellular enzymes and EPS. Thus, there are at least two independent sets of essential pathogenicity functions, one involved in the coordinated production of degradative extracellular enzymes and another which in-

![Fig. 4. Transcriptional activity of the hrp gene(s) mutated by insertion A2 in Xanthomonas campestris pv. campestris grown in MME containing various supplements. β-galactosidase activity is normalized for cell concentration (units as given by Miller 1972; values are means ±1 standard deviation for five independent experiments). Solid bars, supplement only; open bars, supplement with additional 20 mM sucrose.](image-url)
cludes the hpr genes. The contribution of each to the infection process is not yet clear.

We have established that two unrelated phytopathogenic bacteria, which induce different classes of disease and have different host specificities, carry similar hpr gene clusters. This implies that these two pathogens employ some common strategies and mechanisms to attack plants, and also suggests that some of the plant mechanisms involved in HR induction in response to these two pathogens are similar.

Although no homology (by hybridization or complementation) has yet been found between the P. solanacearum-X. campestris hpr cluster and the hpr clusters of other phytopathogenic bacteria, it will be interesting to determine if the different clusters share some common functions.

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LITERATURE CITED


