MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 3, No. 1, pp. 48-53, 1990

A Second Cluster of Genes that Specify Pathogenicity and Host Response in *Pseudomonas solanacearum*

Yong Huang, Peilin Xu, and Luis Sequeira

Department of Plant Pathology, University of Wisconsin, Madison 53706 U.S.A.
Received 15 May 1989. Accepted 17 October 1989.

Two Tn*5*-generated mutants of *Pseudomonas solanacearum* (BOC34B from the nonpathogenic strain B1 and KD688 from the wild-type strain K60) were unable to cause rapid browning or hypersensitive responses (HRs) in callus tissues or leaves, respectively, of the cultivated potato diploid, *Solanum phureja*. In addition, KD688 lost pathogenicity to potato, eggplant, and tobacco. Genetic analyses showed that Tn*5* is inserted in a homologous 7.0-kb *Eco*RI DNA fragment of both mutants. When the cloned Tn*5*-containing fragment from BOC34B, pT34, was marker-exchanged into K60, the resulting strains had the same phenotype of the original mutant and showed the same DNA hybridization pattern as BOC34B. With labeled pT34 as a probe, the corresponding wild-type DNA fragment was identified in a K60 genomic library. When a subclone of this fragment was conjugated into the K60 marker exchange mutants, the Path$^+$ and HR$^+$ phenotypes of the wild-type K60 strain were restored. The DNA region that encodes these functions showed no homology with a previously described *hrp* cluster in *P. solanacearum*. Saturation mutagenesis of the 7.0-kb fragment with Tn*5*-*lac* revealed two separate transcriptional units of at least 1.3 and 0.7 kb. Insertion of Tn*5*-*lac* in either of these units resulted in loss of both pathogenicity and HR functions. When two of these mutants were grown in the presence of plant tissues in either complex or minimal media, expression of the *lac* gene was induced threefold to sixfold above background levels.

Additional keywords: disease resistance, incompatibility, bacterial wilt.

Bacterial wilt, caused by *Pseudomonas solanacearum* E. F. Sm., is one of the most destructive plant diseases worldwide. The bacterium causes wilting of plants belonging to more than 30 families, including many that are economically important crops, such as potato, tobacco, and bananas (Buddenhagen and Kelman 1964). Several studies directed toward the identification of genetic determinants of pathogenicity and host response (hypersensitivity) in *P. solanacearum* have been completed recently (Boucher *et al.* 1988; Schell *et al.* 1988; Xu *et al.* 1988). From this work it is apparent that several genes, designated *hrp* by analogy to similar genes described for other bacterial pathogens (Lindgren *et al.* 1986), control both functions in *P. solanacearum* and appear to be clustered on a very large plasmid carried by all the strains that have been examined (Boucher *et al.* 1987).

Earlier studies concerning the interaction of this pathogen with the cultivated potato diploid, *Solanum phureja* Juz. & Buk., revealed that there are three dominant genes which control resistance of this host to two strains of the bacterium (K60 and S123) (Rowe and Sequeira 1970; Rowe *et al.* 1972; Sequeira and Rowe 1969). Tissue cultures derived from different clones of *S. phureja* show differential reactions (resistance or susceptibility) that correspond to those of the intact plants (Huang *et al.* 1989). Resistance is expressed as a hypersensitivelike response that is characterized by rapid browning and death of the cells. The browning response was shown to be induced by a 60-kDa protein that is produced in large amounts by strain B1 of *P. solanacearum*, particularly when it is grown in contact with callus tissues of clone C-3 of *S. phureja* (Huang *et al.* 1989). Certain Tn*5*-generated mutants of *P. solanacearum*, obtained previously in our laboratory (Morales *et al.* 1985; Xu *et al.* 1988), were found to be affected in pathogenicity and hypersensitive response (HR) induction. We were interested, therefore, in determining whether in these mutants Tn*5* was inserted in the same *hrp* gene cluster described by Boucher *et al.* (1987) or in a different DNA region.

Strain B1 is a spontaneous mutant of the wild-type strain, K60, and is characterized by numerous pleiotropic changes that include loss of pathogenicity and alterations in colony morphology associated with lack of extracellular polysaccharide production (Buddenhagen and Kelman 1964; Kelman 1954; Sequeira 1985). Interestingly, these changes also include acquiring the capacity to induce an HR in a wide variety of hosts, including all clones of *S. phureja* and all cultivars of tobacco that have been tested (Huang *et al.* 1988a; Lozano and Sequeira 1970). The reason for this spontaneous change is unknown, but analysis of restriction fragment polymorphisms of total DNA of strains K60 and B1 does not indicate major DNA rearrangements (D. R. Cook, personal communication). The existence of these two functionally distinct phenotypes provided an opportunity to examine the effects of mutations of both pathogenicity (K60) and hypersensitivity (B1) in very similar genetic backgrounds. In this study, we describe the use of complementation, gene replacement, and saturation mutagenesis to identify a new gene cluster that controls pathogenicity and HR in these particular strains of *P. solanacearum*. The results also indicate that expression of the two transcriptional units in this region may be increased in the presence of plant cells.

© 1990 The American Phytopathological Society

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains and plasmids used in this study are listed in Table 1. Bacteriophage P1::Tn*5-lac* was described by Kroos and Kaiser (1984).

Media and growth conditions. Routinely, *P. solanacearum* strains were cultured in casamino acids-peptone-glucose (CPG) medium (Hendrick and Sequeira 1984) or in TZC medium (Kelman 1954) at 28° C. Mutants were grown in the same media containing appropriate antibiotics. *Escherichia coli* strains were grown on Luria-Bertani (LB) medium (Maniatis *et al.* 1982) at 37° C. For transformation of *P. solanacearum*, the method of Boucher *et al.* (1985) was used.

Growth patterns of *P. solanacearum* strains were determined in potato (clone C-3) callus tissues. Two-week-old calli, approximately 2 g each, were inoculated with 10 μl of bacterial suspension (5×10^7 colony forming units [cfu] per milliliter). At specific intervals, calli inoculated with each strain were comminuted in distilled water, and bacterial populations in the suspension were determined by plate dilution.

Chemicals and reagents. Restriction enzymes were obtained from Promega Biotech (Madison, WI). T4 DNA ligase and a nick translation reagent kit were purchased from Bethesda Research Laboratories (Gaithersburg, MD). Lysozyme, RNase, and chemicals for electrophoresis were from Sigma (St. Louis, MO). Calf intestinal alkaline phosphatase was from Boehringer Mannheim Biochemicals (Indianapolis, IN). Zeta probe membrane was purchased from Bio-Rad (Richmond, CA). Culture media components were from Difco Laboratories (Detroit, MI).

DNA isolation. Total genomic DNA of *P. solanacearum* was extracted and purified from 24–36-hr-old cultures by the method described by Xu *et al.* (1988). In some instances, DNA was further purified by isopycnic centrifugation in CsCl-ethidium bromide.

Molecular techniques. Plasmid purification, agarose gel electrophoresis, restriction nuclease mapping, DNA ligation, bacterial transformation, nick translation, and DNA hybridizations were completed according to standard procedures (Maniatis *et al.* 1982). Colony hybridization was performed on Whatman 541 filter paper by the method of Maas (1983). The plasmid clone (pT34), carrying the Tn*5*-containing *Eco*RI fragment from mutant BOC34B, was isolated previously in our laboratories (Morales *et al.* 1985). The Tn*5*-containing *Eco*RI fragment from mutant KD688 (Xu *et al.* 1988) was cloned in pBR322 to yield the plasmid pK688.

Conjugation of *P. solanacearum*. Four cosmid clones (pL34-A, pL34-B, pL34-C, and pL34-D) were identified from a K60 genomic library by colony hybridization with pT34 as a probe. These four cosmids were individually conjugated into the *P. solanacearum* mutant KT34 by triparental mating (Huang *et al.* 1988b). Transconjugants were purified by restreaking single colonies on TZC medium containing the appropriate antibiotics.

Construction of plasmid pTS34. The four cosmids had unique restriction patterns after digestion with *Eco*RI, but had one DNA band in common. This common fragment

Table 1. Bacterial strains and plasmids used in this study

Designation	Relevant characteristics	Reference or source
Escherichia coli		
DH5α	F^- *endA1 gyrA96 hsdR17* ($r_k^- m_k^+$) *supE44 thi*$^-$ λ^- *recA1 relA1*	Bethesda Research Laboratories, Gaithersburg, MD
TB1	$r_k^- m_k^+$ lacZ(M15)	T. O. Baldwin, Texas A & M, College Station
HB101	*proA2 recA13 hsdS20 ara*($r_k^- m_k^-$) F^- *galK2 rpsL20*(Str) *supE44 leu-6*	Maniatis *et al.* 1982
SF800	*polA thy val*$^+$	Kroos and Kaiser 1984
Pseudomonas solanacearum		
K60	Path$^+$ EPS$^+$ Km^s CM^r	A. Kelman, Univ. of Wisconsin, Madison
B1	Path$^-$ EPS$^-$ Km^s Cm^r	A. Kelman
W70	Path$^+$ EPS$^+$ Km^s Cm^s	D. Thurston, Cornell Univ., Ithaca, NY
W145	Path$^+$ EPS$^+$ Km^s Cm^s	C. Hayward, Univ. Queensland, Brisbane, Australia
KD688	K60::Tn*5* Path$^-$ EPS$^+$ Km^r	This study
BOC34B	B1::Tn*5* Path$^-$ EPS$^-$ HR$^-$ Km^r	Morales *et al.* 1985
KT34	K60::pT34 marker exchange mutant Km^r	This study
KL21	K60::pKL21 marker exchange mutant Km^r	This study
KL15	K60::pKL15 marker exchange mutant Km^r	This study
KL59	K60::pKL59 marker exchange mutant Km^r	This study
KTC34	KT34 carrying pTS34 Km^r Tc^r	This study
Plasmids		
pUC12	Ap^r	Vieira and Messing 1982
pUC18	Ap^r	Vieira and Messing 1982
pBR322	Ap^r Tc^r	Maniatis *et al.* 1982
ColE1::Tn*5*	Km^r	Maniatis *et al.* 1982
pLAFR3	IncP1 Tc^r *rlx*$^+$	Peet *et al.* 1986
pRK2013	IncP Km^r TraRK2$^+$ *repRK2 repE1*$^+$	Stachel *et al.* 1985
pSUP2021	(pBR325 *mob*)::Tn*5* Km^r Ap^r	Simon *et al.* 1983
pT34	pUC12 carrying a 12.9-kilobase (kb) *Eco*RI fragment from BOC34B Ap^r Km^r	Morales *et al.* 1985
pK688	pBR322 carrying a 12.9-kb *Eco*RI fragment from KD688 Ap^r Km^r	This study
pKL21	pUC18 carrying the 7-kb DNA fragment with a Tn*5-lac* insertion	This study
pKL15	pUC18 carrying the 7-kb DNA fragment with a Tn*5-lac* insertion	This study
pKL59	pUC18 carrying the 7-kb DNA fragment with a Tn*5-lac* insertion	This study
pTS34	pLAFR3 carrying the 7-kb DNA fragment	This study
pL34-A, -B, -C, -D	pLAFR3 containing K60 genomic DNA	This study
pL1602	pLAFR3 containing K60 genomic DNA	Xu *et al.* 1988
pVir2	pLAFR3 containing GMI1000 genomic DNA	Boucher *et al.* 1987

(approximately 7 kilobases [kb]) of pL34-A was cut out and eluted as described by Diethard and Renz (1983) and then ligated into *Eco*RI-digested pLAFR3 to yield the plasmid pTS34.

Tn*5-lac* mutagenesis of pTS34. For mutagenesis, the 7-kb *Eco*RI fragment of *P. solanacearum* K60 was cloned into pUC18 and then transformed into *E. coli* DH5α cells. These cells were infected at exponential growth phase with phage P1::Tn*5-lac* at a multiplicity of infection of 0.5–1.0 (Miller 1972). Plasmid DNA from clones with Tn*5-lac* inserted in the 7-kb region was used to transform *P. solanacearum* K60. Expression of the *lacZ* gene in the marker exchange mutants was determined qualitatively on CPG containing X-gal or quantitatively by the *o*-nitrophenyl-β-D-galactoside cleavage method (Miller 1972).

Pathogenicity assays. Eggplant (*Solanum melongena* L. 'Black Beauty') and tobacco (*Nicotiana tabacum* L. 'Bottom Special') seedlings were used for pathogenicity tests, as previously described by Bowman and Sequeira (1982) and Xu *et al.* (1988).

HR assays. To test for hypersensitivity, bacterial suspensions at 1×10^8 cfu/ml were infiltrated into tobacco or potato leaves, as described by Sequeira and Hill (1974). The rapid browning response was tested by direct inoculation of *S. phureja* clone C-3 callus tissue cultures, as described by Huang *et al.* (1989).

RESULTS

Functional and molecular characterization of mutants BOC34B and KD688. The two Tn*5*-insertion mutants, BOC34B from strain B1 and KD688 from strain K60, lost the ability to cause a rapid browning response of potato callus tissues and the HR in potato leaves. In addition, KD688 lost pathogenicity on potato, eggplant, and tobacco. To determine the functional relationships of the Tn*5*-disrupted genes in the two genetic backgrounds (B1 and K60), marker exchange mutagenesis was conducted by transforming strain K60 with the plasmid pT34, which carries the Tn*5*-containing, 12.9-kb *Eco*RI fragment of BOC34B. Fifty independent, kanamycin-resistant transformants were selected and screened for pathogenicity on tobacco and eggplant seedlings and for HR induction in potato leaves and callus tissues. Approximately one half of the transformants failed to cause both wilting and the HR and were sensitive to ampicillin. These transformants appeared to represent double recombination events and one, KT34, was selected for further analysis.

On TZC medium, mutants BOC34B and KT34 grew more slowly than the parental strains, B1 and K60. In potato callus tissues, however, growth rates of the mutants and the parental strains were similar, although BOC34B had a longer lag period than that of B1 (Fig. 1).

Total genomic DNAs of the parent strains K60 and B1, the Tn*5* mutants BOC34B and KD688, and the marker exchange mutant of K60, KT34, were digested with *Eco*RI or *Eco*RI plus *Bam*HI and subjected to agarose electrophoresis. Southern hybridization analysis, with ^{32}P-labeled pT34 as a probe, showed that in mutants BOC34B and KD688, Tn*5* was inserted in the homologous 7.0-kb *Eco*RI fragments of strains B1 and K60. In the marker exchange mutant, KT34, Tn*5* was inserted in the same genomic locus as in the original mutant BOC34B (Fig. 2).

Plasmids pT34 (the Tn*5*-containing *Eco*RI fragment of BOC34B) and pK688 (the Tn*5*-containing *Eco*RI fragment of KD688) were digested with *Eco*RI, *Bam*HI, *Hin*dIII, *Bgl*II, and *Xho*I to determine more precisely the insertion sites of Tn*5*. The results indicate that in the two mutants, Tn*5* is inserted approximately 0.4 kb apart in the same 7.0-kb *Eco*RI fragment (Fig. 3A).

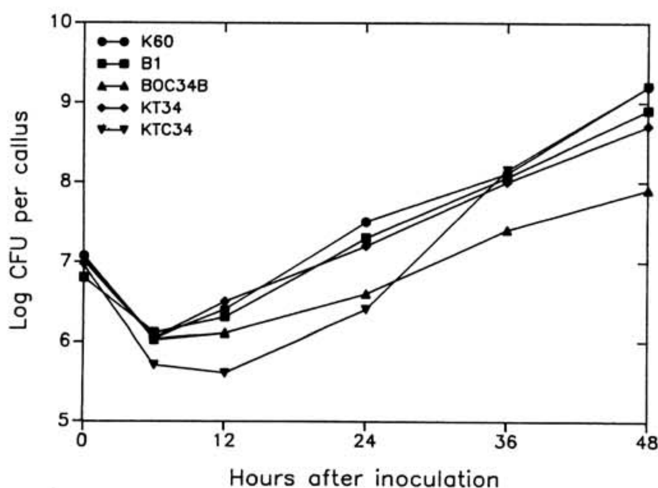

Fig. 1. Growth pattern of *Pseudomonas solanacearum* strains K60 and B1; their respective mutants, KT34 and BOC34B; and the complemented mutant KTC34 in potato (C-3) callus tissues. Two-week-old calli, approximately 2 g each, were inoculated with 10 μl of bacterial suspension (5×10^7 colony forming units [cfu] per milliliter). Results are expressed as colony forming units per callus piece and are the average of three determinations.

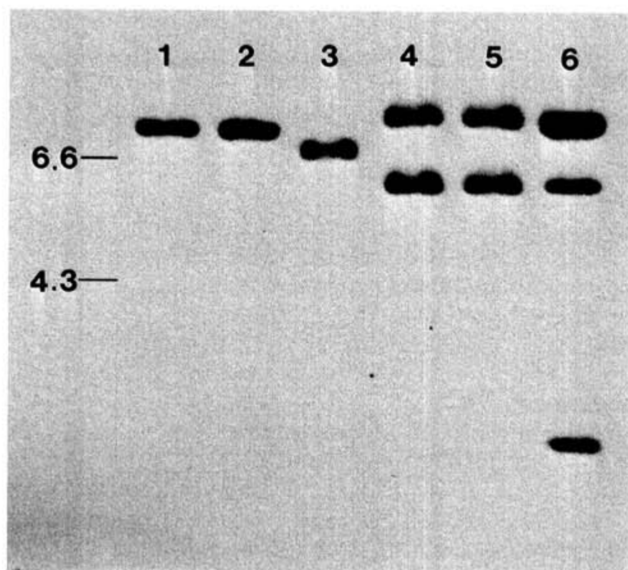

Fig. 2. Southern analysis of DNAs of *Pseudomonas solanacearum* strains K60, B1, KD688, BOC34B, and K60-pT34 transformants (KT34 and KT34A). Total genomic DNA was digested with *Eco*RI (K60 and B1) and *Eco*RI/*Bam*HI (all other strains) and probed with pT34. Lane 1, K60; lane 2, B1; lane 3, KD688 (doublet); lane 4, BOC34B; lane 5, KT34 (double crossover); and lane 6, KT34A (single crossover).

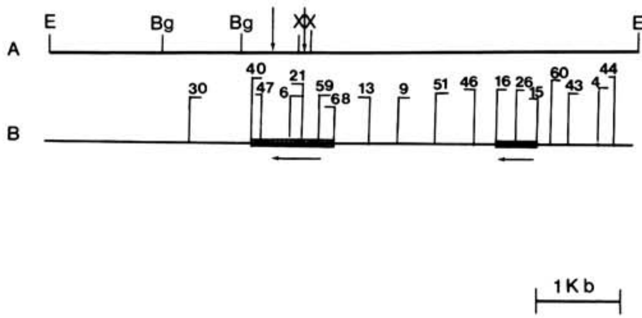

Fig. 3. Saturation mutagenesis of the 7.0-kilobase (kb) *Eco*RI DNA fragment of *Pseudomonas solanacearum* K60 with the transposon Tn*5*-*lac*. **A**, Restriction map of the 7.0-kb fragment. E = *Eco*RI; Bg = *Bgl*II; and X = *Xho*I. The arrow on the left represents the Tn*5* insertion site in mutant BOC34B; the arrow on the right represents the Tn*5* insertion site in mutant KD688. **B**, Pattern of transposon Tn*5*-*lac* insertion sites (bars) in the 7.0-kb *Eco*RI fragment. Orientation of the *lacZ* gene is represented by the flag on top of each bar. The minimum sizes of two transcriptional units are indicated by the broad dark lines; the direction of transcription is indicated by the arrows. Each number represents one Tn*5*-*lac* insertion plasmid (pKL series).

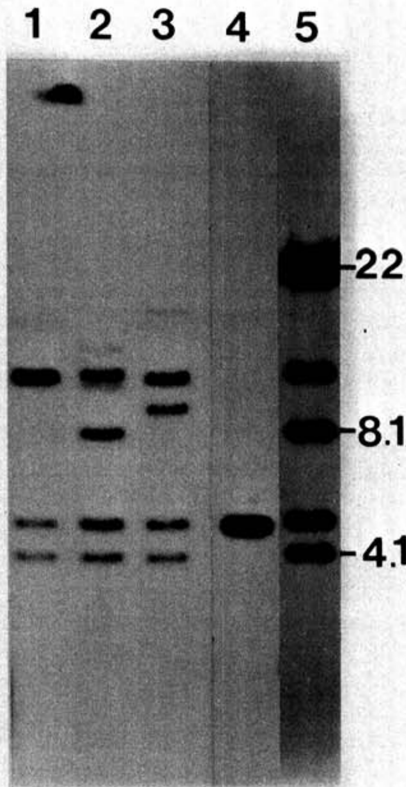

Fig. 4. Southern analysis of total genomic DNAs of *Pseudomonas solanacearum* strains and plasmid DNAs containing inserts of *P. solanacearum* DNA. The blot was probed with ^{32}P-labeled pVir2. Lanes 1–3: *Eco*RI and *Hin*dIII digests of total DNA from strains UW70 (race 2), K60 (race 1), and UW145 (race 3), respectively. Lane 4: *Eco*RI digest of pKD688, a pBR322 clone that contains the 7.0-kilobase (kb) *Eco*RI fragment encompassing the *hrp* region described in this study. Lane 5: *Eco*RI and *Hin*dIII digest of pL1602, an *Eco*RI cosmid clone from K60 that has homology with pVir2. Note that in the digest of pKD688 (lane 4), pVir2 has vector-vector homology with the 4.4-kb band of pBR322, but no homology with the 7.0-kb fragment. In lane 5, vector-vector homology with the 22.0-kb band of pLAFR3 in pVir2 is evident.

As illustrated in Figure 4, pK688 showed no homology by Southern hybridization with pVir2, a clone that contains the cluster of *hrp* genes in strain GMI1000 and is described by Boucher *et al.* (1987).

Complementation of KT34 by cosmid clones. When plasmid pT34 was used as a probe to screen a K60 genomic library, four different cosmid clones (pL34-A, pL34-B, pL34-C, and pL34-D) were identified. Although the four cosmids had different DNA restriction patterns, they shared a common *Eco*RI fragment of approximately 7.0 kb. Southern hybridization analysis confirmed that the four cosmids had a region of homology corresponding to the Tn*5* flanking sequences in pT34 (data not shown).

When the four cosmids were mobilized individually into the marker exchange mutant KT34 by conjugation, all the resulting transconjugants had the Path$^+$, HR$^+$ phenotype of the parental strain, K60. Thus the genes affecting HR and pathogenicity appeared to be located in the common 7.0-kb DNA fragment. To test this assumption, the 7-kb fragment was cloned in pLAFR3 and transferred to strain KT34 by conjugation. As expected, all the transconjugants expressed the functional phenotypes of strain K60, including the ability to cause wilting of eggplant and tobacco seedlings, rapid browning of potato callus tissues, and the HR in potato leaves (Table 2).

Mutagenesis of pTS34. The phage P1::Tn*5*-*lac* was used to introduce Tn*5*-*lac* into *E. coli* DH5α cells carrying the 7.0-kb fragment cloned in pUC18. Eighteen individual clones with Tn*5*-*lac* inserted within the 7.0-kb fragment were isolated, and the site of transposon insertion and the orientation of the *lacZ* gene were determined by analysis of restriction patterns. Two transcriptional units of at least 1.3 and 0.7 kb were identified (Fig. 3B). Marker exchange mutants of strain K60 that had Tn*5* inserted in either transcriptional unit lost pathogenicity as well as the ability to induce the HR. Mutants that had insertions between or outside of the two transcriptional mutants retained the wild-type phenotype (Path$^+$, HR$^+$).

To verify that the marker exchange mutants had acquired Tn*5*-*lac* via a double recombination event, total genomic DNAs from four randomly selected mutants (KL16, KL46, KL47, and KL51) were digested with *Eco*RI and probed with pTS34 by Southern hybridization. The results of this analysis indicated that in these four mutants, transposons

Table 2. Results of bioassays of *Pseudomonas solanacearum* strains for callus browning, hypersensitive response (HR) induction, and pathogenicity

	Browning	HR on		Pathogenicity on	
Strain	**Callus**	**Potato**	**Tobacco**	**Eggplant**	**Tobacco**
K60	BR^{+a}	HR$^+$	N^b	Path$^+$	Path$^+$
B1	BR$^+$	HR$^+$	HR$^+$	Path$^-$	Path$^-$
KD688	BR$^-$	HR$^-$	ND^c	Path$^-$	Path$^-$
BOC34B	BR$^-$	HR$^-$	HR$^-$/HR^{+d}	Path$^-$	Path$^-$
KT34	BR$^-$	HR$^-$	ND	Path$^-$	Path$^-$
KTC34	BR$^+$	HR$^+$	N	Path$^+$	Path$^+$

[a] BR: Rapid browning of potato (*Solanum phureja* C-3) callus tissues.
[b] N: Compatible reaction; necrosis appears after 48 hr.
[c] ND: No reaction for 3–4 days, necrosis appears thereafter.
[d] HR$^-$/HR$^+$: Variable hypersensitive response.

were inserted in the same genomic locus as in the original plasmid (data not shown).

Induction of *hrp* genes by plant tissues. To determine whether expression of the two transcriptional units in the 7.0-kb fragment is induced by plant products, two marker exchange mutants, KL15 and KL21, with Tn*5*-*lac* inserted in the small and large units, respectively, were used. The bacteria were grown to mid-log phase in rich (CPG broth) or minimal A (Miller 1972) media, with or without callus tissues of *S. phureja* clone C-3. Mutant KL59, which has the *lacZ* gene inserted in the wrong orientation and thus is not transcribed, was used as a control. β-galactosidase activity in the supernatant of lysed bacterial cells was measured at specific intervals by the method of Miller (1972). After 2 hr of incubation, the activity of β-galactosidase was threefold to sixfold higher when the KL15 and KL21 mutants were grown in the presence of potato callus tissues than when grown in the absence of callus tissues (Fig. 5). The β-galactosidase activity was similar when bacteria were grown in either rich or minimal media. No β-galactosidase activity was detected when mutant KL59 was grown in the same media, with or without potato callus tissues.

DISCUSSION

In this study, we provide evidence for a cluster of *hrp* genes in *P. solanacearum* that is clearly different from that previously described by Boucher *et al.* (1987). As illustrated in Figure 4, the DNA region that we describe here and which encodes these functions showed no homology with pVir2, a clone encompassing the cluster of *hrp* genes in

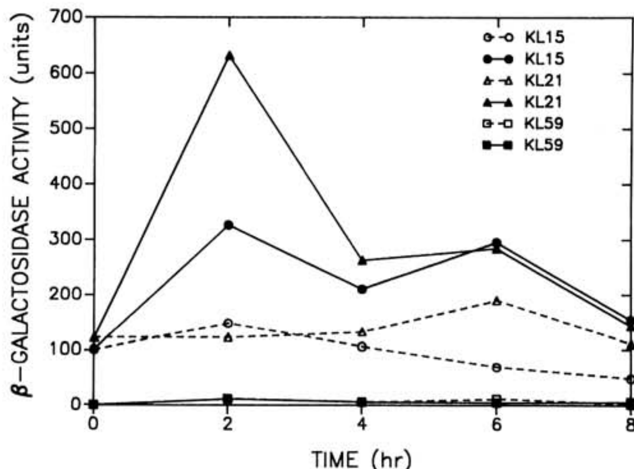

Fig. 5. Induction of the genes in the 7.0-kilobase region of *Pseudomonas solanacearum* K60 by potato (*Solanum phureja* C-3) callus tissues as determined by β-galactosidase activity of Tn*5*-*lac* insertion mutants. The marker exchange mutants, KL15 and KL21, represent the small and the large transcriptional units, respectively. Mutants were grown in casamino acids-peptone-glucose broth with and without callus tissues. Mutant KL59, which contains *lacZ* in the wrong orientation, was used as a control. β-galactosidase activity was measured every 2 hr for 8 hr. Solid lines indicate bacteria cultured with callus tissues; dotted lines indicate bacteria cultured without callus tissues. The activity of β-galactosidase of each mutant was calibrated by the bacterial populations of each mutant. Each point is the average of three replications.

strain GMI1000 and described by Boucher *et al.* (1987) In this new *hrp* region, there are at least two transcriptional units located in a 7.0-kb *Eco*RI fragment of the *P. solanacearum* genome. In the wild-type strain, K60, transposon insertion in either transcriptional unit simultaneously affected the ability to induce the HR in potato and to cause wilting of tobacco. In the strain B1 background, the same mutation affected HR induction in potato and tobacco leaves.

It is significant that a single cosmid (pTS34) complemented the mutations for pathogenicity and HR induction caused by Tn*5*-*lac* insertion in either transcriptional unit. The apparent dual function of HR and pathogenicity genes in the region we have examined indicates that they are part of the *hrp* complex in *P. solanacearum*, but we do not know precisely how they are related physically or functionally to the cluster previously described by Boucher *et al.* (1987). There are, therefore, at least two separate *hrp* clusters in *P. solanacearum*, and these involve several genes that represent either structural or regulatory sequences which control both pathogenicity and HR. From the mutational analysis that we have conducted, we are unable to distinguish between these two possibilities at this time.

Induction of rapid browning of *S. phureja* C-3 callus tissues was not obtained with either mutant BOC34B or KD688, but precisely how this change is associated with the loss of HR induction is not clear at this point. In the single *hrp* mutant we have examined (BOC34B), production in inoculated callus tissues of a 60-kDa protein known to be associated with the rapid browning phenomenon (Huang *et al.* 1989) was reduced significantly when compared with the parental strain (B1).

In our work, the use of a reporter gene (*lac*) fused to a transposable element (Tn*5*) to mutagenize pTS34 allowed analysis of the functional organization of the two transcriptional units in the 7.0-kb region. It has been reported that most plant tissues have a high endogenous activity of β-galactosidase, which would interfere with the use of this enzyme as a marker for gene induction (Jefferson 1987). We did not detect β-galactosidase activity in extracts from potato callus tissues, however, and this allowed the use of Tn*5*-*lac* as a reporter gene system.

Although increased expression of the two transcriptional units that were identified in pTS34 was obtained in response to the presence of plant tissues, the level of induction was moderate (threefold to sixfold) unlike the classical induction pattern (100-fold or greater) for *lac* in *E. coli*, for example, see Miller (1972). Since induction of the *P. solanacearum* genes occurred to a similar extent in both minimal and complex media, it is likely that the transposon-interrupted gene is not subject to glucose-mediated catabolite repression or involved in amino acid transport or incorporation.

The results of the plant tissue induction experiments suggest that, as in the case of *Agrobacterium tumefaciens* virulence (*vir*) genes and *Rhizobium meliloti* nodulation (*nod*) genes (Mulligan and Long 1985; Stachel *et al.* 1986), the *hrp* genes of *P. solanacearum* may be activated by diffusible plant compounds. In preliminary experiments,

which are not reported here, we have determined that induction of the bacterial factor which causes rapid browning of potato callus occurs across a dialysis membrane separating the bacteria from the plant tissues. Thus, these plant factors are of relatively low molecular weight. Work is currently in progress to identify these inducers.

ACKNOWLEDGMENTS

This work was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison (Project 3078), and by grants from the United States Department of Agriculture, the International Potato Center (Lima, Peru), and the National Science Foundation.

The assistance of Douglas R. Cook, who provided some of the Southern analysis data, is gratefully acknowledged. We thank C. Boucher for providing some of the DNA clones used in this work.

LITERATURE CITED

Boucher, C., Arlat, M., Zischek, C., and Boistard, P. 1988. Genetic organization of pathogenicity determinants of *Pseudomonas solanacearum*. Pages 83-95 in: Physiology and Biochemistry of Plant Microbial Interactions. N. Keen, T. Kosuge, and L. Walling, eds. American Society of Plant Physiologists, Bethesda, MD.

Boucher, C., Gijsegem, F., Barberis, P., Arlat, M., and Zischek, C. 1987. *Pseudomonas solanacearum* genes controlling both pathogenicity on tomato and hypersensitivity on tobacco are clustered. J. Bacteriol. 169:5626-5632.

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

Bowman, J., and Sequeira, L. 1982. Resistance to *Pseudomonas solanacearum* in potato: Infectivity titration in relation to multiplication and spread of the pathogen. Am. Potato J. 59:155-164.

Buddenhagen, I., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.

Diethard, T., and Renz, M. 1983. An optimized freeze-squeeze method for the recovery of DNA fragments from agarose gel. Anal. Biochem. 132:14-19.

Hendrick, C. A., and Sequeira, L. 1984. Lipopolysaccharide-defective mutants of the wilt pathogen *Pseudomonas solanacearum*. Appl. Environ. Microbiol. 48:94-101.

Huang, Y., Helgeson, J. P., and Sequeira, L. 1989. Isolation and purification of a factor from *Pseudomonas solanacearum* that induces a hypersensitive-like response in potato cells. Mol. Plant-Microbe Interact. 2:132-138.

Huang, Y., Xu, P. L., and Sequeira, L. 1988a. Use of a potato tissue culture system for molecular analysis of the hypersensitive response (HR) induced by *Pseudomonas solanacearum*. (Abstr.) Phytopathology 78:1610.

Huang, H., Schuurink, R., Denny, T., Atkinson, M. M., Baker, C. J., Yucel, I., Hutcheson, S. W., and Collmer, A. 1988b. Molecular cloning of a *Pseudomonas syringae* pv. *syringae* gene cluster that enables *Pseudomonas fluorescens* to elicit the hypersensitive response in tobacco plants. J. Bacteriol. 170:4748-4756.

Jefferson, R. A. 1987. Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep. 5:387-405.

Kelman, A. 1954. The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

Kroos, L., and Kaiser, D. 1984. Construction of Tn*5 lac*, a transposon that fuses *lacZ* expression to exogenous promoters, and its introduction into *Myxococcus xanthus*. Proc. Natl. Acad. Sci. USA 81:5816-5820.

Lindgren, P. B., Peet, R. C., and Panopoulos, N. J. 1986. Gene cluster of *Pseudomonas syringae* pv. "*phaseolicola*" controls pathogenicity on bean plants and hypersensitivity on nonhost plants. J. Bacteriol. 168:512-522.

Lozano, J. C., and Sequeira, L. 1970. Differentiation of races of *Pseudomonas solanacearum* by a leaf infiltration technique. Phytopathology 60:833-838.

Maas, R. 1983. An improved colony hybridization method with significantly increased sensitivity for detection of single genes. Plasmid 10:296-298.

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

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

Morales, V., Stemmer, W., and Sequeira, L. 1985. Genetics of avirulence in *Pseudomonas solanacearum*. Pages 89-96 in: Current Communications in Molecular Biology: Plant Cell/Cell Interaction. I. Sussex, A. Ellingboe, M. Crouch, and R. Malmberg, eds. Cold Spring Harbor, NY.

Mulligan, J. T., and Long, S. R. 1985. Induction of *Rhizobium meliloti nodC* expression by plant exudate requires *nodD*. Proc. Natl. Acad. Sci. USA 82:6609-6613.

Peet, R. C., Lindgren, P. B., Willis, D. K., and Panopoulos, N. J. 1986. Identification and cloning of genes involved in phaseolotoxin production by *Pseudomonas syringae* pv. *phaseolicola*. J. Bacteriol. 166:1095-1105.

Rowe, P. R., and Sequeira, L. 1970. Inheritance of resistance to *Pseudomonas solanacearum* in *Solanum phureja*. Phytopathology 60:1499-1501.

Rowe, P. R., Sequeira, L., and Gonzalez, L. C. 1972. Additional genes for resistance to *Pseudomonas solanacearum* in *Solanum phureja*. Phytopathology 62:1093-1094.

Schell, M., Roberts, D., and Denny, T. 1988. Analysis of the *Pseudomonas solanacearum* polygalacturonase encoded by *pglA* and its involvement in phytopathogenicity. J. Bacteriol. 170:4501-4508.

Sequeira, L., and Rowe, P. R. 1969. Selection and utilization of *Solanum phureja* clones with high resistance to different strains of *Pseudomonas solanacearum*. Am. Potato J. 46:451-462.

Sequeira, L., and Hill, L. M. 1974. Induced resistance in tobacco leaves: The growth of *Pseudomonas solanacearum* in protected tissues. Physiol. Plant Pathol. 4:447-455.

Sequeira, L. 1985. Surface components involved in bacterial pathogen-plant host recognition. J. Cell Sci. Suppl. 2:301-316.

Simon, R., Priefer, U., and Puhler, A. 1983. A broad host range mobilization system for *in vitro* genetic engineering: Transposon mutagenesis in gram negative bacteria. Biotechnology 1:781-790.

Stachel, S. E., An, G., Flores, C., and Nester, E. W. 1985. A Tn*3 lacZ* transposon for the random generation of β-galactosidase gene fusions: Application to the analysis of gene expression in *Agrobacterium*. EMBO J. 4:891-898.

Stachel, S. E., Nester, E. W., and Zambryski, P. C. 1986. A plant cell factor induces *Agrobacterium tumefaciens vir* gene expression. Proc. Natl. Acad. Sci. USA 83:379-383.

Vieira, J., and Messing, J. 1982. The pUC plasmid, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259-268.

Xu, P., Leong, S., and Sequeira, L. 1988. Molecular cloning of genes that specify virulence in *Pseudomonas solanacearum*. J. Bacteriol. 170:617-622.