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Transcriptional Organization and Expression of the Large *hrp* Gene Cluster of *Pseudomonas solanacearum*

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Cloning and localized mutagenesis of the larger cluster of *hrp* genes of *Pseudomonas solanacearum* strain GMI1000 allowed the definition of the borders of this cluster, which now extends about 2 kb to the left of the insert of the previously described plasmid pVir2 (Boucher *et al.* 1987, J. Bacteriol. 169:5626-5632). The size of the cluster has also been expanded 3 kb to the right to include a region previously described as *dsp*; our present data demonstrate that insertions occurring in these 3 kb lead to leaky mutations affecting both pathogenicity on tomato and ability to induce the hypersensitive response (HR) on tobacco. Therefore, the size of the entire *hrp* gene cluster is estimated to be about 22 kb. The use of transposon Tn*5*-B20, which promotes transcriptional gene fusions, allowed us to demonstrate that the *hrp* gene cluster is organized in a minimum of six transcriptional units, which are transcribed when the culture is grown in minimal medium but are repressed during growth in rich medium or in the presence of peptone or Casamino Acids. The level of expression in minimal medium is modulated by the carbon source provided; pyruvate is the best inducer. Under these conditions the level of expression observed *in vitro* appears to be representative of the actual expression observed *in planta*.

Additional keywords: bacterial wilt, plant pathogen.

Plant pathogenic bacteria have the ability to invade and to multiply within certain plants, causing typical disease symptoms. A particular bacterium usually infects only a limited number of plant species. On nonhost plants, disease does not develop. It has been shown that this is often correlated with the development of a hypersensitive response (HR), rapid necrosis of plant tissue that has come into contact with the pathogenic bacterium (Klement and Goodman 1967). Among bacteria, the ability to induce the HR is strictly restricted to plant pathogens, suggesting that certain pathogenicity determinants might be recognized by the plant and act as HR inducers. Genetic evidence favors the existence of such determinants common to both processes. Mutants resulting from a single mutational event have been isolated that are affected in both pathogenicity and HR-inducing ability. Genes governing such properties have been called *hrp* genes and have been found in different species including *Pseudomonas syringae* van Hall (Anderson and Mills 1985; Lindgren *et al.* 1986, Huang *et al.* 1988; Cuppels 1986), *Erwinia amylovora* (Burrill) Winslow *et al.* (Barny *et al.* 1990, Beer *et al.* 1991), and, more recently, *Xanthomonas campestris* (Pammel) Dowson (Kamoun and Kado 1990; Bonas *et al.* 1991; Arlat *et al.* 1991). Current knowledge about *hrp* genes has recently been reviewed (Willis *et al.* 1991).

P. solanacearum (Smith) Smith *hrp* genes have been identified at two different loci (Boucher *et al.* 1986; Huang *et al.* 1990), and we have already partly delimited the large *hrp* gene cluster (Boucher *et al.* 1987), which we had shown to be located on the megaplasmid present in most strains of *P. solanacearum* (Rosenberg *et al.* 1982). This gene cluster has been shown to be conserved among all virulent strains of the bacterium independently of host or geographical origin (Boucher *et al.* 1988).

In this paper we present additional data on the size and transcriptional organization of the large *hrp* gene cluster together with the role of different factors involved in the regulation of the expression of these genes. A preliminary report of part of this work has been presented elsewhere (Arlat *et al.* 1990).

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MATERIALS AND METHODS

Biological material and culture conditions. Bacterial strains, plasmids, phages, and transposon used in this study are listed in Table 1. *Escherichia coli* strains were grown at 37° C in LB broth (Maniatis *et al.* 1982) and *P. solanacearum* strains were grown at 30° C in B, BG, BGT (Boucher *et al.* 1986), or MM (Boucher *et al.* 1988) media. MM was supplemented with various carbon sources used at 10 mM final concentration. Antibiotics were used at the following concentrations (mg L^{-1}): tetracycline (Tc), 10; kanamycin (Km), 30; streptomycin (Sm), 200; nalidixic acid (Nal), 50; and ampicillin (Amp), 50.

Tn*5*-B20 mutagenesis. Mutagenesis using phage lambda573::Tn*5*-B20 was performed as previously described (Arlat *et al.* 1991). Mapping of the insertions was performed by analysis of the restriction fragments from minipreps.

Bacterial conjugation. All conjugations were performed on plates with 10^9 donor and 10^9 recipient cells. When required, triparental matings were performed using the helper strain *E. coli* K12 carrying the plasmid pRK2013 (Figurski and Helinski 1979). Unless otherwise stated, matings were performed overnight at 30° C on BG plates followed by selection of the transconjugants on appropriate

plates. Bacteriophage T4 was used to counterselect *E. coli* in interspecific matings.

Transformation of *P. solanacearum*. Transformation of strain GMI1000 leading to homologous recombination of the incoming DNA was performed as previously described (Boucher *et al.* 1985), except that the plasmids used for transformation were extracted from *E. coli* using the alkaline lysis procedure. Before transformation, pBS2.2 derivatives were linearized by digestion at the unique *Xba*I restriction site present in the vector.

Molecular biology techniques. Standard methods were used for plasmid extraction, restriction fragment analysis, and DNA cloning (Maniatis *et al.* 1982). Southern blots of DNA restriction fragments were performed on Biodyne (Pall, NY) membranes according to the manufacturer's instructions. Radioactive DNA probes were obtained through random oligo priming (Feinberg and Vogelstein 1983).

β-Galactosidase assays. Unless otherwise stated, *P. solanacearum* strains carrying Tn*5*-B20 insertions were grown overnight until late exponential phase in B broth. Cells were then collected by centrifugation, washed twice with deionized water, and resuspended in water to an OD_{600} of 2.0–2.5. This suspension was used as inoculum and diluted 1 to 20 in fresh medium and grown for 14 hr. β-Galactosidase activity was determined by measuring the production of *O*-nitrophenol from *O*-nitrophenyl-β-*O* galactoside (ONPG) according to Miller (1972), except that the volumes were scaled down to fit into Eppendorf tubes. The units are expressed according to Miller's definition. Each measure was performed in triplicate.

To measure *hrp* gene expression *in planta*, GMI1000 derivatives carrying Tn*5*-B20 insertion in the *hrp* gene cluster were inoculated into tobacco or tomato plants.

Tobacco leaves were infiltrated with strains carrying insertions 1484 or 1487. Whereas insertion 1484 does not affect the Hrp phenotype, insertion 1487 leads to a Hrp$^-$ phenotype. Aqueous suspensions of bacteria were infiltrated into undetached leaves with a syringe. Inoculated plants were incubated at 30° C under light. After 8 hr, before appearance of the collapse, inoculated leave were detached and infiltrated under vacuum with deionized water. Bacteria present in the intercellar fluid were then collected by low-speed centrifugation. The recovered bacterial pellet was washed twice with Z buffer (Miller 1972) and used for β-galactotosidase assay. To estimate the level of β-galactosidase from plant origin, control experiments were performed inoculating plant with water or strain GMI1000, which is devoid of β-galactosidase activity.

To assess expression of *hrp* genes in the compatible host, 1-mo-old tomato plants were stem-inoculated with 100 μl of an aqueous suspension of mutant GMI1484 (10^9 cells/ml). The inoculated plants were kept at 30° C with 16 hr light, 8 hr dark until the appearance of the early wilt symptoms (3–4 days). Three-centimeter-long stem segments were collected 1 cm above and below the inoculation point in order to exclude the bacteria present close to the inoculation point and to recover bacteria that had actually diffused away in the plant. The collected stem segments were incubated for 2–3 hr in 5 ml of Z buffer to allow bacteria to ooze out of the infected tissues. Bacteria were then collected by centrifugation and washed twice in Z buffer to eliminate potential β-galactosidase activity of plant origin. The final pellet was then resuspended in Z buffer (Miller 1972) and assayed for activity.

For each plant species the experiment was performed with two plants. This was independently repeated twice.

Pathogenicity assays. Pathogenicity on axenic tomato seedlings and ability to induce the HR following infiltration into tobacco leaf parenchyma were assayed as previously described (Boucher *et al.* 1985).

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

Strain	Relevant characteristics	Reference or source
Bacteria		
Pseudomonas solanacearum		
GMI1000	Wild-type	Boucher *et al.* 1985
Escherichia coli		
K12	Wild-type	R. Devoret
C600	*thi*-1, *thr*-1, *thr*-1, *leu*B6, *lac*Y1, *ton*A21, *sup*E44	Maniatis et al. 1972
C2110 Nal^r	*pol*A1, *rha, his,* Nal^r	Leong *et al.* 1982
S17-1	RP4(Tc::Mu)(Km::Tn*7*) inserted into the chromosome, Sm^r	Simon *et al.* 1989
Plasmids		
pBluescript KS$^-$	Ampicillin resistance	Stratagene
pVir2	pLAFR3 carrying part of the *hrp* gene cluster	Boucher *et al.* 1987
pAFE8	pLAF3 carrying part of the *hrp* gene cluster	This paper
pBS2.2	pBluescript KS$^-$ with 8-kb *Eco*RI fragment from pVir2	This paper
pRK2013	*tra*RK2, *ori*ColE1	Figurski and Helinski 1979
Transposon		
Tn*5*-B20	Km^r, promotorless *lacZ*	Simon *et al.* 1989
Phages		
T4	Specific for *E. coli*	R. Devoret
lambda573	*b221(att,int),* red$^-$, *O*am, *P*am, cI857	N. Kleckner

RESULTS

Delimitation of the *hrp* gene cluster. Saturation mutagenesis of plasmid pVir2 had previously suggested that the *hrp* gene cluster extends beyond the left-hand end of the pVir2 insert, since all the transposon insertions located at the left-hand end of this insert resulted in a Hrp$^-$ mutant phenotype (Boucher *et al.* 1987). Therefore we used the 4-kb *Eco*RI restriction fragment located at the left end of the pVir2 insert to screen a previously constructed genomic bank of strain GMI1000 (Boucher *et al.* 1987) for overlapping clones extending further on the left side. This led to the isolation of cosmid pAFE8, which carries a 25-kb insert extending 20 kb to the left of pVir2.

Plasmids pAFE8, pVir2, and the pBluescriptKS$^-$ derivative pBS2.2, which carries the 8-kb *Eco*RI fragment from pVir2, were mutagenized by insertion of transposon Tn*5*-B20. This transposon confers resistance to kanamycin and generates transcriptional gene fusions between the promotor of the target gene and the *E. coli* β-galactosidase coding sequence (Simon *et al.* 1989). This mutagenesis

resulted in a set of insertions that mapped at least every kilobase along the pVir2 and pAFE8 region.

Each insertion was then individually marker-exchanged into the *P. solanacearum* wild-type strain GMI1000. This was done by transformation of the recipient strain with linearized pBS2.2 derivatives or by conjugation with transposon Tn*5*-B20-carrying derivatives of the cosmid clones. Transposon-carrying *P. solanacearum* derivatives were selected using kanamycin. In all cases Km^r transformants or exconjugants strains were found not to have acquired the antibiotic resistance encoded by the vector plasmid, suggesting that acquisition of the transposon resulted from homologous recombination or from transposition. This is similar to the situation previously reported for the mutagenesis of pVir2 (Boucher *et al.* 1987).

The genomic structure of selected exconjugants and transformants was analyzed by hybridizing *Bam*HI, *Eco*RI, and/or *Hin*dIII genomic blots of each strain with either a pVir2 or a pAFE8 probe. Contrary to what had been observed using the Tn*5-lac* of Kroos and Kaiser (1984), all the clones obtained resulted from a marker exchange process and in no case was transposition detected. We observed that transformation with *Xba*I-linearized plasmid always resulted in a marker exchange event with the recipient strain, whereas various events leading to integration of the vector plasmid were observed when transformation was performed using circular DNA molecules.

Each of the recombinant strains carrying a Tn*5*-B20 insertion was tested for pathogenicity on axenic tomato seedlings and for the HR-inducing ability in tobacco leaves. In all cases a strict correlation was found between these two properties; all the mutants affected for one property were also affected for the other. Figure 1 shows the site of each insertion together with the corresponding phenotype (hereafter, the Tn*5*-B20 insertion mutants of strain GMI1000 will be designated by the letters GMI followed by the number of the insertion they carry). All the *hrp* mutants mapped in a continuous stretch of DNA, demonstrating that the entire gene cluster spans over about 22 kb of DNA. This region extends about 2 kb to the left-hand end of the pVir2 insert, and its left border is located between insertions 1459 and 1462. None of the insertions

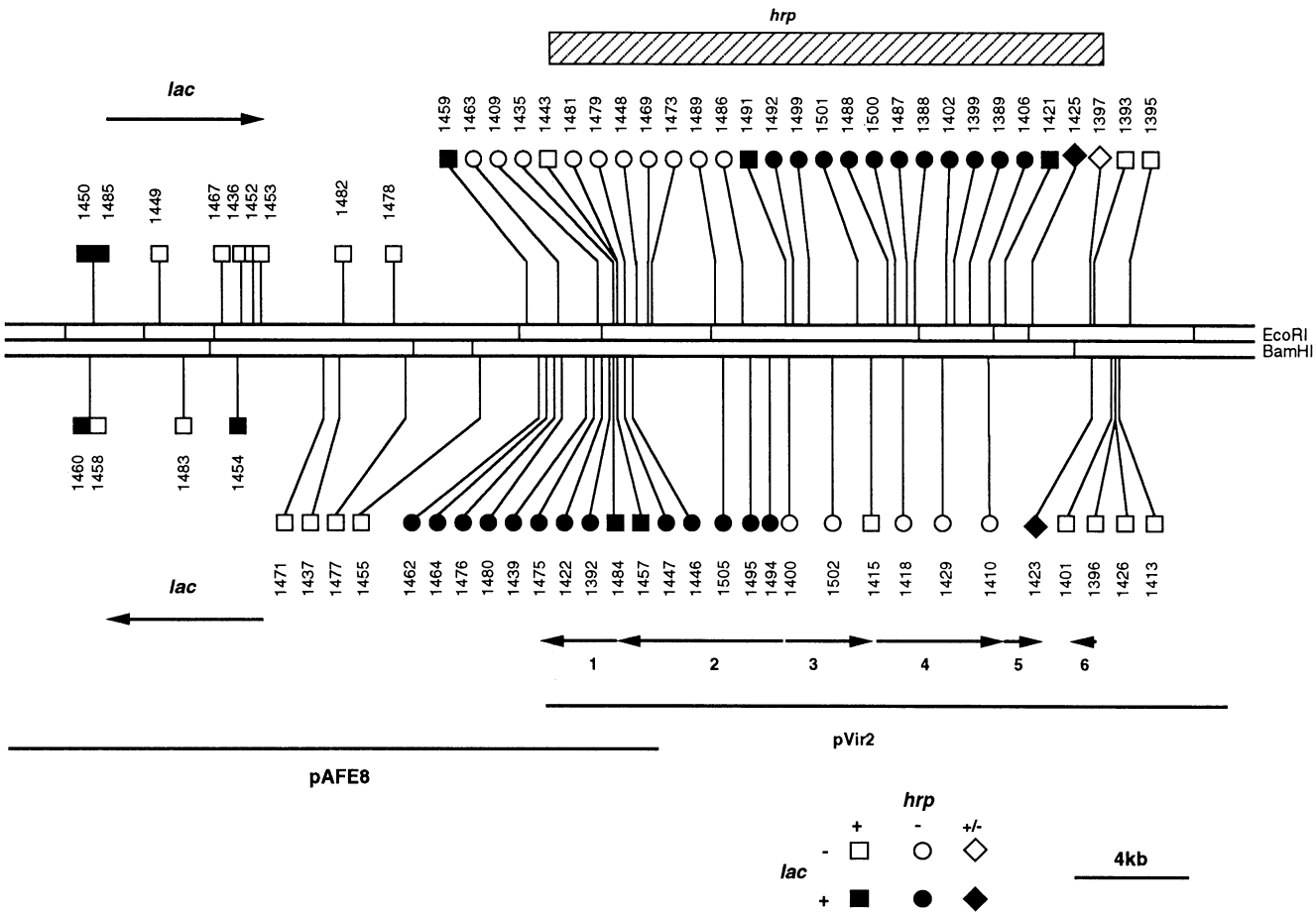

Fig. 1. Physical and genetic map of the large *hrp* gene cluster of *Pseudomonas solanacearum* GMI1000. The DNA inserts carried by plasmids pAFE8, pVir2, and pBS2.2 are represented by bars below the restriction map. Tn*5*-B20 insertion sites are shown by vertical bars. Insertions resulting in an Hrp^- phenotype are indicated by circles placed on top of the bars; insertions leading to a wild-type phenotype are represented by squares. Diamonds on top of bars show insertions resulting in leaky Hrp phenotype. All the insertions represented above the map correspond to the ones in which the orientation of the transposon is such that transcription of the *lacZ* coding sequence should occur from left to right, whereas insertions presented below the map are in the reverse orientation. Insertions that resulted in a significant β-galactosidase activity are represented with closed symbols; open symbols correspond to insertions with no significant activity. Arrows below the map correspond to putative transcriptional units. Abbreviations: E, *Eco*RI; B, *Bam*HI.

located to the left of insertion 1459 affected the pathogenic properties of the wild-type strain. Most of the insertions located between insertions 1462 and 1421 totally abolished the HR-inducing ability and plant pathogenicity. Insertions 1423, 1425, and 1397 led to leaky mutants that still induced limited disease symptoms on axenic tomato plants. These mutants were also leaky for the HR-inducing activity, since from one experiment to the other they induced a delayed HR and/or an HR that only affected part of the infiltrated tissues or no reaction at all. This region in which insertions led to a leaky phenotype had previously been described as a *dsp* region (for *d*isease *sp*ecific) (Boucher *et al.* 1987). In light of the present data, we propose that the entire region located between the insertion sites 1459 and 1393 should be considered as the *hrp* gene cluster. Six insertions within this cluster did not result in a mutant phenotype. These insertions map at three different sites within the *hrp* gene cluster. For each of these insertions the presence of the transposon at the corresponding site was confirmed by Southern hybridization of genomic blots. Because of the polar effect of transposon insertion it is assumed that these insertions occurred in intercistronic regions of the *hrp* gene cluster or very close to the distal end of individual transcriptional units, although other possibilities could be considered.

Transcriptional organization of the *hrp* gene cluster. Because strain GMI1000 is naturally devoid of β-galactosidase activity and because Tn*5*-B20 promotes transcriptional gene fusions with the coding sequence of the reporter gene *lacZ*, insertions obtained within the *hrp* gene cluster and in the flanking regions were used to monitor *hrp* gene expression. Preliminary experiments performed on a limited number of insertions had established that there were no large differences in β-galactosidase activities measured at different stages of the growth curve of the bacteria (data not shown). Therefore for each insertion, β-galactosidase activity was measured following growth for 20 hr in MM + glucose until mid-exponential phase (OD_{600}: 0.4 to 0.8). Table 2 presents the average values of at least two independent measurements performed on each mutant. Under these conditions about half of the insertions resulted in production of a low level of activity (<10 units), whereas for the others this activity varied from 15 to more than 300 units. Under the same conditions strictly no activity was detected in the wild-type strain.

Based both on orientation of the insertions that expressed more than 10 units of β-galactosidase activity and on the position of insertions within the *hrp* gene cluster that led to a wild-type phenotype, a minimum of six putative transcriptional units within this gene cluster could be defined. These are shown in Figure 1 and Table 2.

Regulation of *hrp* gene transcription. For most individual recombinant strains carrying a Tn*5*-B20 insertion within the *hrp* gene cluster, β-galactosidase activity was also measured following growth for 20 hr in rich medium until late exponential phase. Data from a single experiment presented in Table 2 show that expression of the entire *hrp* gene cluster is repressed in these conditions. For mutant GMI1487 (carrying insertion 1487) that produces a significant level of β-galactosidase activity in MM + glucose, β-galactosidase activity was also measured following

Table 2. β-Galactosidase activity (Miller's units) of strain GMI1000 derivatives carrying a Tn*5*-B20 insertion in the *hrp* gene cluster and in the flanking regions

Transcriptional unit	GMI number of the mutants[a]	Tn5-B20 orientation[b]	β-Galactosidase activity	
			MM + glucose	B
	1450	L/R	317	
	1485	L/R	3101	103
	1460	R/L	182	
	1458	R/L	25	
	1449	L/R	3	
	1483	R/L	2	
	1467	L/R	2	1
	1454	R/L	69	13
	1436	L/R	2	
	1452	L/R	3	
	1453	L/R	2	
	1471	R/L	1	
	1437	R/L	2	
	1482	L/R	3	
	1478	L/R	1	
	1477	R/L	4	
	1455	R/L	2	1
	1459	L/R	50	4
	1462	R/L	65	2
	1464	R/L	55	
	1476	R/L	31	2
	1480	R/L	44	2
	1439	R/L	26	2
unit 1	1475	R/L	24	4
	1422	R/L	55	10
	1392	R/L	32	10
	1463	L/R	7	
	1409	L/R	4	3
	1435	L/R	1	1
	1443	L/R	2	
	1484	R/L	190	
	1457	R/L	200	8
	1481	R/L	2	1
	1479	R/L	2	
unit 2	1447	R/L	366	4
	1446	R/L	344	9
	1505	R/L	95	4
	1495	R/L	237	6
	1494	R/L	314	
	1469	R/L	10	4
	1473	R/L	10	4
	1489	R/L	3	2
	1486	R/L	4	3
	1491	L/R	97	3
	1492	L/R	174	7
	1499	L/R	52	2
unit 3	1501	L/R	14	6
	1400	R/L	2	2
	1502	R/L	3	2
	1415	R/L	8	3
	1488	L/R	40	
	1500	L/R	123	
	1487	L/R	98	3
	1388	L/R	40	3
	1402	L/R	98	3
unit 4	1399	L/R	35	2
	1389	L/R	39	2
	1406	L/R	105	3
	1418	R/L	15	3
	1429	R/L	2	
	1410	R/L	9	
	1421	L/R	261	8
unit 5	1425	L/R	80	
unit 6	1397	L/R	1	2
	1428	R/L	138	
	1401	R/L	3	3
	1396	R/L	2	2
	1326	R/L	3	2
	1413	R/L	2	3
	1393	L/R	1	3
	1395	L/R	5	2

[a] Insertions are ordered according to their relative position on the mutagenized area.

[b] Orientation of the transposon: L/R, *lacZ* oriented from left to right; R/L reverse orientation.

growth in MM + glucose supplemented with all the ingredients of B broth at their habitual concentrations (Fig. 2). Under these conditions no expression was observed, suggesting that B broth repressed *hrp* gene transcription. Similar repression was observed when MM + glucose was supplemented with Difco Casamino Acids at 10 g L^{-1} (Fig. 2).

Because it has already been reported in other systems that expression of *hrp* genes is dependent on the carbon source provided for growth (Fellay *et al.* 1991; Arlat *et al.* 1991), β-galactosidase produced by mutant GMI1487 was measured following growth in MM supplemented with various carbon sources, each provided at a final concentration of 10 mM. Substrates were chosen that can be metabolized by strain GM1000 and by comparison with carbon sources used in similar studies performed with other plant pathogenic bacteria. As shown in Figure 2, depending on the carbon source provided, the level of β-galactosidase activity varied 49-fold. Bacteria grown in the presence of pyruvate showed the highest activity, whereas growth with glycerol, fructose, and sorbitol gave the lowest activities. However, these were significantly higher than those observed when bacteria were grown in B broth or in MM supplemented with glucose and Casamino Acids.

***hrp* gene expression *in planta*.** A strain of *P. solanacearum* carrying the insertion 1484 is pathogenic on tomato and induces an HR on tobacco. This insertion probably maps at the 3′ end of the putative transcriptional unit 2 because it is transcribed from right to left and expressed β-galactosidase activity at a level similar to that of the gene fusions belonging to unit 2. In addition, expression of this gene fusion is regulated by the carbon source in a similar fashion to GMI1487 (data not shown). The β-galactosidase activity was measured for strains GMI1484 and GMI1487 following inoculation into tomato or tobacco plants. This was compared with activity found in these strains grown in MM supplemented with pyruvate. Data shown in Table 3 indicate that a comparable expression of these gene fusions occurs *in planta* and *in vitro*. This suggests that the level of expression of *hrp* genes observed in MM + pyruvate is representative of the level of expression of these genes in tomato and tobacco. This conclusion can probably be extended to the entire *hrp* gene cluster because in similar experiments performed once in tobacco using mutants GMI1462, GMI1475, GMI1447, GMI1492, GMI1399, GMI1425, and GMI1423, which carry insertions mapping to each putative transcriptional unit, the level of β-galactosidase activity obtained in MM + pyruvate was comparable or slightly superior to the level measured *in planta* (data not shown). Control experiments using GMI1000, which is devoid of β-galactosidase activity, to inoculate plants established that no contaminating activity from plant origin was recovered under the assay conditions used (Table 3).

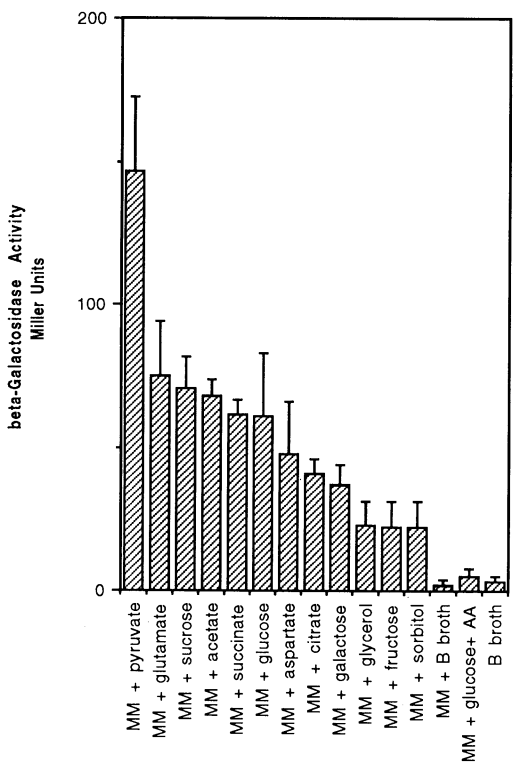

Fig. 2. Effect of different carbon sources on transcriptional activity of *hrp* gene(s). Mutant GMI1487 was grown for 14 hr in MM supplemented with various carbon sources or in B broth before β-galactosidase activity was measured as described by Miller (1972). Values are the average of two independent experiments. Bars correspond to standard errors. Abbreviation: AA, Casamino Acids.

DISCUSSION

We had previously described the existence of a cluster of *hrp* genes in *P. solanacearum*, part of which was shown to be present on plasmid pVir2. Isolation of plasmid pAFE8, which carries an insert partly overlapping with the left-hand end of pVir2, followed by transposon mutagenesis of this plasmid showed that the left border of the *hrp* gene cluster is located about 2 kb to the left of the region carried on plasmid pVir2. Moreover, isolation of additional mutants in the right-hand end of the pVir2 insert demonstrated that the 3-kb region located to the right-hand end of the pVir2 insert and which was previously thought to be exclusively involved in disease development, is in fact also required for the normal development of the HR. Therefore we propose that this region should be included in the *hrp* gene cluster, although mutations in this region lead to a reduced response following inoculation on both tomato and tobacco plants. The extended cluster thus defined spans a region of about 22 kb and is therefore very similar in size to the *hrp* gene clusters of *P. syringae* (Rahme *et al.* 1991) and *X. campestris* (Bonas *et al.* 1991;

Table 3. Comparison of the expression of *hrp* genes in pyruvate (10 mM) containing MM with the expression in compatible (tomato) and incompatible (tobacco) hosts

	Bacterial β-galactosidase activity[a] in		
Strains	**MM + pyruvate[b]**	**Tomato**	**Tobacco**
GMI1484	105	61	96
GMI1487	98	NT[c]	87
GMI1000	<2	NT	<2

[a] Miller's units, values are the means of at least two independent experiments.
[b] Activity measured after 8 hr of growth.
[c] Not tested.

Arlat *et al.* 1991) and to the *hrp* and "*xrp*" regions of *E. amylovora* defined, respectively, by Barny *et al.* (1990) and Beer *et al.* (1991).

The use of transposon Tn*5*-B20 permitted the definition of a minimum of six putative transcriptional units in the *P. solanacearum hrp* gene cluster. It is possible that certain of these units actually correspond to two or more operons transcribed in the same orientation and determination of individual complementation units through genetic analysis would resolve this question. Unfortunately, such analysis has not been performed since it was not possible to construct and/or maintain stable merodiploid strains carrying either pAFE8 or pVir2. This suicide behavior of pVir2 had been previously observed in *P. solanaceurum* (Boucher *et al.* 1987) and in *X. campestris* pv. *campestris* (Arlat *et al.* 1991). It is probably due to the presence of particular gene(s) present on the inserts of pAFE8 and pVir2 since the vector plasmid pLAFR3 can be stably transferred and maintained in the wild-type *P. solanacearum* strain GMI1000.

Recently, the *hrp* gene cluster of *X. c.* pv. *vesicatoria* was shown to be organized in a minimum of six complementation groups (Bonas *et al.* 1991). Although we had previously shown the existence of DNA homologies between *P. solanacearum hrp* genes and *X. c.* pv. *vesicatoria* genomic DNA (Boucher *et al.* 1987) and the colinearity between *P. solanacearum* and *X. c.* pv. *campestris hrp* gene clusters (Arlat *et al.* 1991), it is not presently possible to draw any conclusion concerning the relationship between transcriptional units defined for *P. solanacearum* and complementation groups of *X. c.* pv. *vesicatoria.* On the other hand, the transcriptional organization of the *P. solanacearum* cluster is clearly different from that which has been established for *P. syringae* pv. *phaseolicola* (Rahme *et al.* 1991).

The present work establishes that *P. solanacearum hrp* genes expression is induced in MM and repressed in rich medium. It is not known whether this repression resulted from the presence of a particular amino acid or if it was due to the supply of a high level of organic nitrogen. Alternatively, this effect could result from a change in osmolarity, although growth in double-strength MM did not affect gene expression (data not shown). A similar regulation pattern depending on the nutritional status of the bacteria has been reported in all *hrp* gene clusters so far investigated: *P. s.* pv. *phaseolicola* (Rahme *et al.* 1991), *E. amylovora* (Beer *et al.* 1991), and *X. c.* pv. *campestris* (Arlat *et al.* 1991).

It should be noted that in the present work the expression of five gene fusions resulting from insertions 1450, 1460, 1454, 1485, and 1459 which map outside of the *hrp* gene cluster followed the same regulation pattern. This raised the question of whether the regulation observed for *hrp* genes is specific for this class of genes or whether it resulted from a more general regulation circuit. A similar situation has been observed for the *vir* region of *Agrobacterium tumefaciens* (Smith and Townsend) Conn. In this organism, expression of the *vir* genes that are essential for tumorogenicity is induced in the presence of acetosyringone (Stachel *et al.* 1985). These genes are flanked to the left by the *pinF* locus, which is coregulated with the *vir* genes but which does not play an essential role in pathogenicity (Stachel and Nester 1986). It has been proposed that *pinF* might specifically function during the plant-bacteria interaction (Stachel and Nester 1986). The same might be true for the *P. solanacearum* genes adjacent to the *hrp* gene cluster if the regulation observed *in vitro* for these genes actually reflects the regulation occurring *in planta.* The identification of *pehA*, a gene encoding an extracellular polygalacturonase, located adjacent to the left end of the *hrp* gene cluster in strain K60 (Allen *et al.* 1991) could support this hypothesis.

During growth in MM the level of transcription of *P. solanacearum hrp* genes can be modulated by the carbon source supplied. Pyruvate is the best substrate; glycerol, fructose, and sorbitol were the three poorest tested. Although the nature of the carbon source has already been reported to modulate the level of expression of *hrp* genes in *X. c.* pv. *campestris* (Arlat *et al.* 1991) and of the *hrp* dependent *avrB* gene in *P. s.* pv. *glycinea* (Huynh *et al.* 1989), it is interesting to note some gross differences in the activity of particular substrates in the different organisms. Fructose, which is the best substrate in *P. s.* pv. *glycinea* has very little activity in *P. solanacearum*; on the other hand pyruvate, which is very efficient in *P. solanacearum,* ranks lowest in *X. c.* pv. *campestris* and *P. s.* pv. *glycinea.* In contrast, sucrose appears to be a fairly good substrate in all three organisms, reinforcing the hypothesis proposed by Huynh *et al.* (1989) that sucrose, which is very abundant in leaf tissues, contributes to expression of pathogenicity-related genes during infection.

We have also shown that the level of expression of *P. solanacearum hrp* genes observed during growth in MM supplemented with pyruvate is comparable to the level of expression of these genes observed following inoculation into plants. This suggests that the regulation of *hrp* genes observed *in vitro* actually reflects the gene regulation occurring *in planta.* However, conclusive evidence in favor of this assumption will come from the identification and study of regulatory genes involved in the control of *hrp* gene expression.

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