MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 5, No. 5, pp. 390-396, 1992

Determinants of Pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are Related to Proteins Involved in Secretion in Bacterial Pathogens of Animals

Stefan Fenselau, Ilse Balbo, and Ulla Bonas

Institut für Genbiologische Forschung Berlin GmbH, Ihnestr. 63, 1000 Berlin 33, Germany.
Received 11 June 1992. Accepted 7 July 1992.

One of the model systems investigated for studying plant bacterial pathogenesis is *Xanthomonas campestris* pv *vesicatoria*, the causal agent of bacterial spot disease of pepper and tomato. Genes necessary for both basic pathogenicity and the induction of the hypersensitive response in resistant plants (*hrp* genes) were previously isolated from *X. c.* pv. *vesicatoria* and characterized genetically. As a first step toward functional analysis, part of the *hrp* gene cluster, making up several loci, was sequenced. Here, we report the first indications of the function of *hrp* genes. Striking similarities to proteins from the mammalian pathogens *Shigella flexneri*, *Yersinia enterocolitica*, *Y. pestis*, and other bacteria were discovered. Proteins encoded by genes within the *X. c.* pv. *vesicatoria* loci *hrpA*, *hrpB*, and *hrpC* are similar to ATPases and to *Yersinia* Ysc and LcrD proteins, which are involved in secretion of Yop proteins, a particular class of essential pathogenicity factors produced by *Yersinia* species. This finding indicates, for the first time, that the fundamental determinants of pathogenicity may be conserved among bacterial pathogens of plants and animals. We hypothesize that *hrp* genes are involved in the secretion of molecules essential for the interaction of *X. c.* pv. *vesicatoria* with the plant.

Additional keyword: virulence.

A major objective in the investigation of pathogenic microbes is the elucidation of the basic mechanism underlying parasitism and the incitement of disease. Whereas animal pathogenic bacteria produce toxins and adhesins that play an essential role in pathogenicity, comparatively little is known about the biochemical mechanisms involved in basic pathogenicity of bacteria that are pathogens of plants (Daniels *et al.* 1988; Finlay and Falkow 1989). The interaction between bacterial plant pathogens and their hosts requires the concerted action of a large number of bacterial functions. Genes necessary for basic pathogenicity in susceptible plants and for the induction of the hypersensitive response (HR) in resistant plants were operationally defined as *hrp* genes and have been isolated from a number of gram-negative phytopathogenic bacteria, including different subspecies of *Pseudomonas*, *Xanthomonas*, and *Erwinia* (recently reviewed by Willis *et al.* 1991).

The *hrp* genes in *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, the causal agent of bacterial spot disease of pepper and tomato, map to a 25-kb chromosomal region (Bonas *et al.* 1991). Mutations in any one of the six *hrp* loci abolish any readily detectable interaction with the plant (e.g., growth in the intercellular space of the plant tissue and development of typical disease symptoms in susceptible host plants). In addition, mutations in *hrp* genes also prevent induction of the HR in resistant hosts or nonhost plants (Bonas *et al.* 1991). Although there are several examples of cross hybridization between cloned *hrp* genes from a number of different species of phytopathogenic bacteria (Boucher *et al.* 1987; Arlat *et al.* 1991; Bonas *et al.* 1991), genetic interspecies complementation of *hrp* loci has only been observed between related subspecies (Bonas *et al.* 1991; U. Bonas, unpublished).

We previously demonstrated that *X. c.* pv. *vesicatoria* *hrp* gene expression is plant-inducible and suppressed in complex culture media (Schulte and Bonas 1992). A similar pattern of regulation has been described for *hrp* genes in pathovars of *Pseudomonas syringae* van Hall (Lindgren *et al.* 1989; Rahme *et al.* 1991, 1992; Xiao *et al.* 1992), *P. solanacearum* (Smith) Smith (Arlat *et al.* 1992), and *X. c.* pv. *campestris* (Pammel) Dowson (Kamoun and Kado 1990; Arlat *et al.* 1991), and recently for *Erwinia amylovora* (Burrill) Winslow *et al.* (Wei *et al.* 1992). Except for the regulatory genes *hrpRS* and *hrpL* from *P. s.* pv. *phaseolicola* (Burkholder) Young *et al.* (Grimm and Panopoulos 1989; Fellay *et al.* 1991), the biochemical functions of *hrp* genes in *X. c.* pv. *vesicatoria* or any other phytopathogenic bacterium have not been elucidated. As a first step in addressing this question, we are sequencing the entire *hrp* region from *X. c.* pv. *vesicatoria*. Here, we report the analysis of three *hrp* loci. We have discovered striking similarities between Hrp proteins and proteins that are involved in the secretion of pathogenicity factors by the mammalian pathogens *Shigella flexneri* Castellani and Chalmers, *Yersinia enterocolitica* (Schleifstein and Coleman) Frederiksen, and *Y. pestis* (Lehmann and Neumann) Van Loghem, the causal agents of dysentery, gastroenteritis, and bubonic plague, respectively. Our findings have led us to propose a working hypothesis for *hrp* gene function.

Address correspondence to Ulla Bonas.

The protein sequences of HrpA1, HrpB3, HrpB6, and HrpC2 have been submitted to SwissProt with the accession numbers P80151, P80152, P80153, and P80153, respectively.

MATERIALS AND METHODS

Cloning procedures. *Eco*RI, *Eco*RV, and *Bam*HI fragments of the cosmid clone pXV9 (Bonas *et al.* 1991; Fig. 1) were cloned into pBluescript II KS from Stratagene (La Jolla, CA) to obtain overlapping subclones. For sequence analysis, deletion clones of appropriate subclones were generated by the use of DNaseI, as previously described (Bonas *et al.* 1989). Standard molecular techniques were used (Maniatis *et al.* 1982).

Sequence analysis. The DNA sequence of both strands of overlapping deletion subclones of pXV9 was determined by the dideoxy chain termination method (Sanger *et al.* 1977). Double-stranded templates were sequenced with commercial primers (Stratagene) or custom primers and T7 DNA polymerase (Pharmacia, Uppsala, Sweden). The DNA sequence was analyzed with the University of Wisconsin GCG package (Version 7.0; Devereux *et al.* 1984). We found putative open reading frames (ORFs) with a high probability for expression by determining the codon preference (CODONPREFERENCE; Devereux *et al.* 1984) of the DNA sequences. The amino acid sequences of the deduced polypeptides encoded by *hrpA*, *hrpB*, and *hrpC* were compared with sequences present in the GenBank database by the use of the program TFASTA (Devereux *et al.* 1984). Pairwise alignments were performed by BESTFIT analysis (Devereux *et al.* 1984); the following parameters were applied: gap weight of 3.0 and length weight of 0.1. Multiple sequence alignments were done by a combination of BESTFIT, LINEUP (Devereux *et al.* 1984), and visual inspection. We performed hydropathy analyses and searches for signal sequences by using SOAP and PSIGNAL, respectively, from the PC/Gene analysis package (IntelliGenetics Inc., University of Geneva, Switzerland).

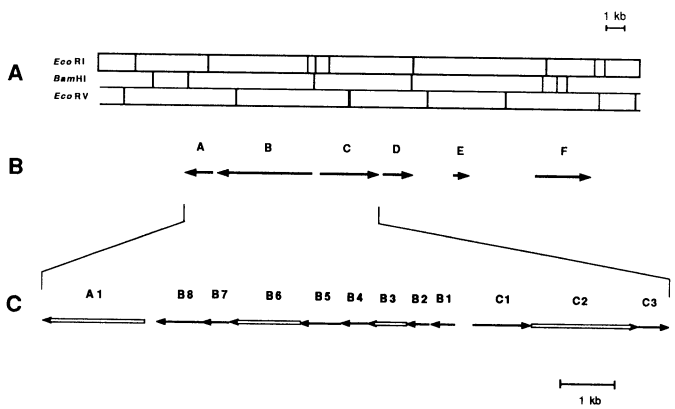

Fig. 1. Structural organization of the *hrp* region in *Xanthomonas campestris* pv. *vesicatoria*. **A,** The restriction map of the entire *hrp* region (Bonas *et al.* 1991) is shown for *Eco*RI, *Bam*HI, and *Eco*RV. **B,** The arrows indicate position and orientation of the *hrp* loci, designated *hrpA–hrpF* (see Bonas *et al.* 1991; Schulte and Bonas 1992). The sizes of the loci *hrpD–hrpF* were deduced from the genetic analysis. The sizes of *hrpA–hrpC* are based on a combination of genetic and sequence analyses. **C,** The region corresponding to *hrpA–hrpC* was sequenced and analyzed for possible coding regions. Relative position and size of the deduced open reading frames (ORFs) are represented by the arrows. The ORFs were designated *hrpA1* in *hrpA*, *hrpB1–hrpB8* in *hrpB*, and *hrpC1–hrpC3* in *hrpC*. The larger arrows refer to sequences with significant similarity to known proteins (see text).

RESULTS AND DISCUSSION

DNA sequence analysis. The physical map of the cloned *X. c.* pv. *vesicatoria hrp* region and of the positions of the complementation groups *hrpA–hrpF* is shown in Figure 1. The region that is of interest for this study is in the left portion of the *hrp* cluster, present in the insert of plasmid pXV9 (Bonas *et al.* 1991; Fig. 1A). Appropriate restriction fragments from pXV9 were subcloned into pBluescript. Unidirectional deletion clones, generated from overlapping subclones in both orientations, were subsequently sequenced. A more detailed analysis and the complete nucleotide sequence of the *hrp* loci will be published elsewhere. With our previous results from transposon mutagenesis, complementation studies, and also from *hrp* gene expression studies with Tn*3-Gus* insertions in this region (Bonas *et al.* 1991; Schulte and Bonas 1992), we deduced the following structural organization. The 10-kb region shown in Figure 1C makes up the left end of the *hrp* cluster containing the loci *hrpA*, *hrpB*, and *hrpC*. We analyzed the sequence that contains a number of ORFs for potentially translated ORFs by using the computer program CODONPREFERENCE (Devereux *et al.* 1984). Those ORFs predicted to be expressed were designated *hrpA1*, *hrpB1–hrpB8*, and *hrpC1–hrpC3*. Genetic data suggest that the locus *hrpA* probably consists of a single ORF, *hrpA1* (Bonas *et al.* 1991). Because *hrpB* and *hrpC*

Table 1. Similarity between Hrp proteins from *Xanthomonas campestris* pv. *vesicatoria* and proteins from other bacteria

Protein	Length (amino acids)	Bacterium	Protein similarity score[a]	Localization and function[b]	Reference
HrpA1	607	*Yersinia enterocolitica*	YscC (34/55)	Periplasm or OM[c]; Yop[d] secretion	Michiels *et al.* 1991
HrpB3	253	*Y. enterocolitica*	YscJ (34/56)	OM; lipoprotein; Yop secretion	Michiels *et al.* 1991
HrpB6	442	*Bacillus subtilis*	FlaA-ORF4 (50/68)	Cytoplasm; ATPase	Albertini *et al.* 1991
		Salmonella typhimurium	FliI (48/65)	Cytoplasm; ATPase	Vogler *et al.* 1991
		Shigella flexneri	Spa47 (45/64)	Cytoplasm; ATPase	Venkatesan *et al.* 1992
		Escherichia coli	β-su F1 ATPase (30/53)	ATPase subunit	Saraste *et al.* 1981
HrpC2	645	*Y. pestis*	LcrD (46/70)	IM[e]; regulator of Yop secretion	Plano *et al.* 1991
		Caulobacter crescentus	FlbF (35/55)	IM; regulator of flagellum apparatus	Ramakrishnan *et al.* 1991; Sanders *et al.* 1992

[a] The similarity scores are given as percentage of identity/similarity on the basis of sequence comparisons with BESTFIT (Devereux *et al.* 1984).
[b] Based on data given in the cited references.
[c] Outer membrane.
[d] *Y*ersinia *o*uter membrane *p*roteins.
[e] Inner membrane.

contain several ORFs that, based on codon preference analysis, are likely to be expressed, we conclude that these genes are organized in operons. This is confirmed by our analyses of transposon insertions in this region (Bonas *et al.* 1991).

Sequence comparison of the putative *hrp* gene products with known sequences. The amino acid sequence of all deduced polypeptides encoded by the *hrpA*, *hrpB*, and *hrpC* loci (Fig. 1C) were compared with sequences present in the GenBank data bank. We found striking similarities between some of the putative Hrp proteins and proteins in bacteria that are not pathogens of plants. These data are summarized in Table 1 and strongly indicate that the predicted ORFs are translated into polypeptides.

HrpA1. The predicted HrpA1 protein is 64 kDa. The N-terminal sequence resembles a signal peptide sequence, with basic residues at position 10–12, a hydrophobic core sequence, and a putative cleavage site between positions 33 and 34. The presence of one transmembrane domain (amino acid positions 260–277) suggests a localization of this region in the membrane. A search in the data banks revealed that HrpA1 is 34% identical (55% similar) to the YscC protein, which is encoded by the *virC* operon of *Y. enterocolitica* (Michiels *et al.* 1991), a human pathogen that causes gastroenteritis (Fig. 2). The same similarity was found to the KIM5 gene product of *Y. pestis* (Genbank accession M83225; P. L. Haddix and S. C. Straley, unpublished), which is 98% identical to YscC from *Y. enterocolitica*. The *Yersinia* YscC protein also contains an N-terminal signal peptide sequence, suggesting transport of the protein across the inner membrane. Although HrpA1 and the YscC protein have about the same length, the last 100 amino acids in the C-terminal region of YscC show no similarity to HrpA1. In contrast to the structural organization of the *virC* genes in *Y. enterocolitica* (Michiels *et al.* 1991), the *hrpA* locus consists solely of *hrpA1*.

Weaker sequence similarities were found between regions of HrpA1 and the following proteins: 24% identity and 49% similarity (allowing 20 gaps) to the PefD protein of *X. c.* pv. *campestris*, which is involved in secretion of extracellular enzymes (Hu *et al.* 1992); and 23% identity (46% similarity) to PulD from *Klebsiella pneumoniae* (Schroeter) Trevisan (d'Enfert *et al.* 1989). The PulD protein is important for secretion of pullulanase and has been reported to be in the outer membrane.

HrpB3. For *hrpB*, two putative proteins were found to share sequence similarity with proteins in the data bank. HrpB3 is a putative 27.3-kDa protein (Fig. 3) with a hydrophobic core in the amino-terminal region followed by a perfect lipoprotein signal sequence cleavage site for signal peptidase II at position Cys19 (Leu-Ser-Ala-Cys; Wu and Tokunaga 1986). The presence of two possible membrane-spanning hydrophobic domains suggests that part of HrpB3 might be localized in the membrane. The HrpB3 protein sequence shows 34% identity to the 27-kDa lipoprotein YscJ in *Y. enterocolitica* (Michiels *et al.* 1991; Fig. 3). Notably, YscJ is encoded by the same operon as YscC, the *virC* operon (Michiels *et al.* 1991). The YscJ and HrpB3 proteins share an overall similarity of 56%,

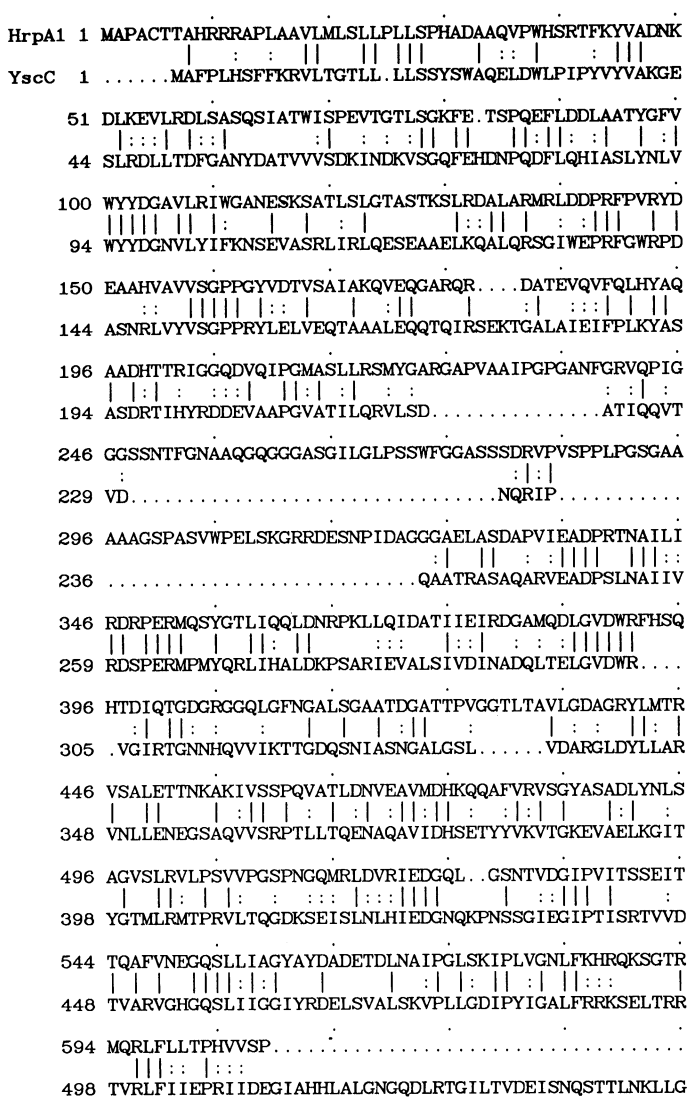

Fig. 2. Comparison of deduced amino acid sequence of HrpA1 from *Xanthomonas campestris* pv. *vesicatoria* and YscC from *Yersinia enterocolitica* (Michiels *et al.* 1991). The sequences were aligned by BESTFIT (Devereux *et al.* 1984). From YscC (608 amino acids), only the first 448 amino acids are shown. Identical amino acids are indicated with vertical bars; conserved exchanges are indicated with colons. Gaps are represented by dots.

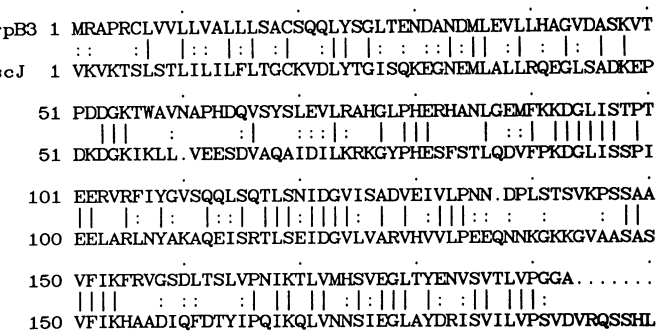

Fig. 3. Sequence alignment between the deduced amino acid sequences of HrpB3 from *Xanthomonas campestris* pv. *vesicatoria* and YscJ from *Yersinia enterocolitica* (Michiels *et al.* 1991). See also Figure 2.

but the C-terminal regions of the proteins are less well conserved.

HrpB6. The putative HrpB6 protein is 47.7 kDa and predicted to be hydrophilic. The amino acid sequence of HrpB6 is related to FlaA-ORF4 (50% identical, 68% similar) from *Bacillus subtilis* Mendelson (Albertini *et al.* 1991) and to FliI from *Salmonella typhimurium* (Loeffler) Castellani and Chalmers (Vogler *et al.* 1991; Table 1 and Fig. 4). Furthermore, the HrpB6 sequence shares 45% identity and 64% similarity with the putative Spa47 protein from *S. flexneri*, which causes bacillary dysentery (Venkatesan *et al.* 1992). All four proteins are nearly identical in length, and the amino acid sequence conservation is not simply restricted to certain domains but is found throughout their entire lengths. HrpB6 as well as FlaA-ORF4, FliI, and Spa47 are approximately 30% identical to the β-subunit of ATPases from different bacteria (e.g., from *Escherichia coli* (Migula) Castellani and Chalmers) (Saraste *et al.* 1981). The highest conservation between the HrpB6, FlaA-ORF4, FliI, Spa47, and ATPase β-subunit proteins is found in the mononucleotide (Fig. 4, boxes A and B; Walker *et al.* 1982) and the magnesium (box C; Yoshida *et al.* 1982) binding domains described for ATPases, suggesting that they have a similar function (Fig. 4).

HrpC2. By far the most significant sequence similarity of an Hrp protein to a protein of *Yersinia* was found for HrpC2, which has six possible transmembrane domains

Fig. 4. Comparison of deduced amino acid sequence of HrpB6 and related proteins. HrpB6 from *Xanthomonas campestris* pv. *vesicatoria*, FliI from *Salmonella typhimurium* (Vogler *et al.* 1991), FlaA-ORF4 from *Bacillus subtilis* (Albertini *et al.* 1991), and the β-subunit of the *Escherichia coli* F1-ATPase (Saraste *et al.* 1981) were aligned by BESTFIT. Box A and Box B indicate the nucleotide-binding regions; Box C indicates the magnesium-binding region of the ATPase (Walker *et al.* 1982; Yoshida *et al.* 1982). Capital letters in the consensus sequence that was performed by LINEUP refer to identical residues in all four sequences. The dots fill gaps for optimal alignment.

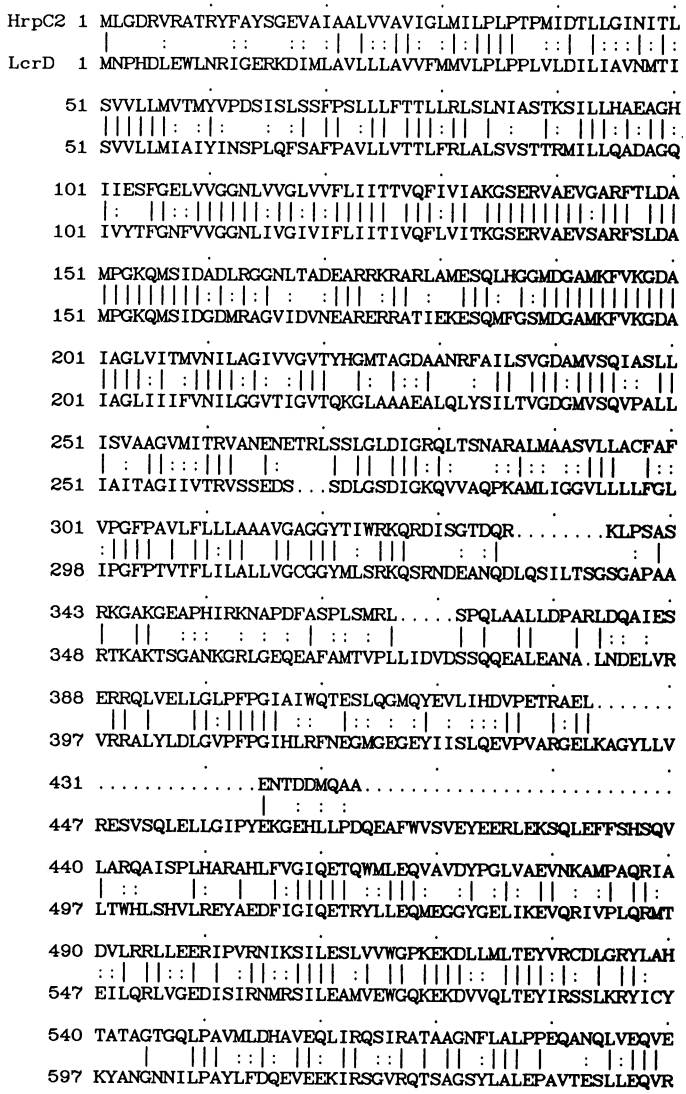

Fig. 5. Sequence alignment between the deduced amino acid sequences of HrpC2 from *Xanthomonas campestris* pv. *vesicatoria* and LcrD from *Yersinia pestis* (Plano *et al.* 1991). See Figure 2 for details.

in the amino-terminal half of the 69.9-kDa protein. The protein does not appear to possess a signal sequence. We found high similarity of HrpC2 to LcrD from *Y. pestis* (Plano *et al.* 1991) and to the LcrD homolog in *Y. enterocolitica*, the sequence of which is incomplete (Viitanen *et al.* 1990). The HrpC2 and LcrD proteins display 46% identity and 70% similarity throughout their entire lengths (645 and 704 amino acids, respectively; Fig. 5); the middle region, however, is less well conserved. In *Yersinia*, the analysis of fusion proteins between LcrD and PhoA has shown that the amino-terminal domain of LcrD protein is localized in the inner membrane of the cell, whereas the C-terminal domain of LcrD seems to be localized in the cytoplasm (Plano *et al.* 1991). The similarity between HrpC2 and LcrD is not simply a result of the presence of transmembrane domains in both proteins. Comparisons with other proteins that have transmembrane domains, such as the tetracycline resistance protein (Hillen and Schollmeier 1983), did not reveal significant similarity. Furthermore, HrpC2 is similar to several other predicted proteins from different bacteria. We found 39% identity (65% similarity) to MxiA from *S. flexneri* (G. P. Andrews and A. T. Maurelli, unpublished; GenBank accession M91664); the *mxiA* gene is located upstream of the *spa* operon, and part of its sequence was recently published (Venkatesan *et al.* 1992). HrpC2 is 37 and 35% identical (63 and 55% similar) to FlhA from *B. subtilis* (P. B. Carpenter and G. W. Ordal, unpublished; GenBank accession X63698) and to FlbF *Caulobacter crescentus* Poindexter, respectively (Ramakrishnan *et al.* 1991; Sanders *et al.* 1992) (see Table 1).

Conclusions and working hypothesis. The striking similarities of 40–50% identity and up to 70% similarity between Hrp proteins from *X. c.* pv. *vesicatoria* and proteins from *Yersinia*, *S. flexneri*, *B. subtilis*, *S. typhimurium*, and *C. crescentus* suggest that these proteins are related and might perform similar functions. The same observations were made for putative Hrp proteins from *Pseudomonas solanacearum* (Smith) Smith, HrpA, HrpI, and HrpO, (Gough *et al.* 1992); these proteins turned out to be highly similar to the HrpA1, HrpB3, and HrpC2 proteins from *X. c.* pv. *vesicatoria* discussed here. To date, other than the *hrpS* gene in *P. s.* pv. *phaseolicola*, which probably plays a regulatory role in *hrp* gene expression (Grimm and Panopoulos 1989), the function of *hrp* genes of *X. c.* pv. *vesicatoria* or any other phytopathogenic bacterium is unknown.

In addition to the strong similarities between individual proteins that are determinants of pathogenicity in plant and animal pathogens there is, clearly, similarity in the overall organization of the corresponding genes. Thus, like the *hrp* loci in *X. c.* pv. *vesicatoria*, genes encoding the Hrp-related proteins in *Yersinia* and *Shigella* are also genetically linked. The *mxiA* and *spa* genes in *S. flexneri* are located in a 37-kb region on a large plasmid and are essential for bacterial invasion of host cells (Hale 1991). The *Yersinia* proteins described above are encoded by the *vir* (virulence) and *lcr* (*l*ow *c*alcium *r*esponse) operons that are localized on a 70-kb plasmid that is required for virulence (Cornelis *et al.* 1989). Mutations in either YscC, YscJ, or LcrD have pleiotropic effects. Most notably, secretion of the Yop proteins (*Yersinia* *o*uter membrane *p*roteins), which are essential virulence factors encoded by genes on the same plasmid, is prevented in such mutants (Goguen *et al.* 1984; Michiels *et al.* 1991). It has been postulated, therefore, that the *lcr* and *vir* genes play a role in secretion of the Yop proteins and/or in the regulation of the Yop secretory pathway (Cornelis *et al.* 1989; Michiels *et al.* 1991; Plano *et al.* 1991). In contrast to most proteins that are secreted from bacteria, the Yop proteins lack a typical signal peptide sequence and, therefore, require a specific secretion pathway (Michiels and Cornelis 1991). The same is true for the Ipa proteins (*i*nvasion *p*lasmid *a*ntigens) from *S. flexneri* (Hale 1991). Specific transport pathways have been described for other secreted proteins lacking a classical signal peptide sequence, such as proteases from *Erwinia chrysanthemi* Burkholder *et al.* (Delepelaire and Wandersman 1989) and hemolysin from *E. coli* (Felmlee *et al.* 1985). Because the *Yersinia* proteins LcrD and possibly YscC and YscJ are membrane proteins, they might be part of the Yop secretion apparatus. A regulatory role in Yop secretion has been suggested for the LcrD protein (Plano *et al.* 1991), but in *X. c.* pv. *vesicatoria* there is no indication that *hrpC2* is a regulatory gene (T. Horns and U. Bonas, unpublished). HrpC2 and LcrD might differ in this respect, because part of the C-terminal half of the proteins is clearly distinct (Fig. 5).

We postulate that some of the Hrp proteins make up part of a secretion system that is required for pathogenicity of *Xanthomonas*. HrpA1, HrpB3, and HrpC2 could be part of such a specific transport apparatus. In addition to the similarity to the *Yersinia* YscC protein, most of the HrpA1 sequence is similar to other proteins associated with secretion (e.g., PefD from *X. c.* pv. *campestris* [Hu *et al.* 1992] and PulD from *K. pneumoniae* [d'Enfert *et al.* 1989]). HrpB6 is structurally and possibly functionally related to ATPases. Because HrpB6 from *X. c.* pv. *vesicatoria* is the only protein in the *hrpB* operon showing conservation to ATPases, we postulate that HrpB6 functions as an ATPase that is related to a transport apparatus, rather than as part of a proton pump. This proposal is supported by the finding that a number of ATP-binding proteins are involved in specialized secretion systems, such as Prt in *Erwinia* and Hly in *E. coli* (Higgins *et al.* 1986). Furthermore, the HrpB6-related proteins, FlaA-ORF4 (Albertini *et al.* 1991) and FliI (Vogler *et al.* 1991), are postulated to play a role in export of flagellum proteins, whereas the Spa47 protein from *S. flexneri* is essential for transport and surface presentation of the Ipa proteins (Venkatesan *et al.* 1992). Although these ATPase-related proteins are hydrophilic and most likely localized in the cytoplasm, they could be associated with proteins in the inner membrane.

Although it is generally assumed that there must be trafficking across the bacterial membrane(s) during the interaction with the host, the nature of the molecules transported is unknown. There are several possibilities. One is the secretion of degradative enzymes that contribute to the provision of better nutritional conditions in the intercellular space of the plant tissue. Secretion of extracellular enzymes has been shown to be involved in pathogenicity of *X. c.* pv. *campestris*, but the corresponding genes of their secretory pathway (Dums *et al.* 1991) are not localized

in the *hrp* region and are not homologous to the Hrp proteins described here. Degradative enzymes such as pectate lyase secreted by *X. c.* pv. *campestris* do not seem to have a major role in the pathogenicity of *X. c.* pv. *vesicatoria* (Beaulieu *et al.* 1991). It may be, therefore, that *X. c.* pv. *vesicatoria* expresses and secretes, like yersiniae (Cornelis *et al.* 1989), additional and so far unknown virulence factors. What could be the nature of these factors in *X. c.* pv. *vesicatoria*? Preliminary experiments have not revealed any obvious differences in the patterns of proteins secreted by wild-type and *hrp* mutant *X. c.* pv. *vesicatoria* bacteria (S. Fenselau, unpublished). This is in contrast to the copious amounts of Yop proteins secreted by *Yersinia* (Heesemann *et al.* 1986; Michiels and Cornelis 1990).

Regarding induction of the hypersensitive response, the molecules secreted via the Hrp-derived transport apparatus are attractive candidates for elicitors of the plant's response (Fig. 6). In the *X. c.* pv. *vesicatoria*-pepper interaction, bacterial avirulence genes control induction of the HR in pepper cultivars carrying a corresponding resistance gene. None of the products of the avirulence genes characterized so far contain signal sequences. One of the avirulence genes studied in our laboratory is the *avrBs3* gene (Bonas *et al.* 1989). The *avrBs3* gene constitutively expresses a soluble protein, but the HR does not develop if the bacteria lack *hrp* functions (Knoop *et al.* 1991). We hypothesize that avirulence proteins, such as the AvrBs3 protein (or part of it) or Avr-derived specific elicitors, are secreted by the proposed transport apparatus as outlined in Figure 6.

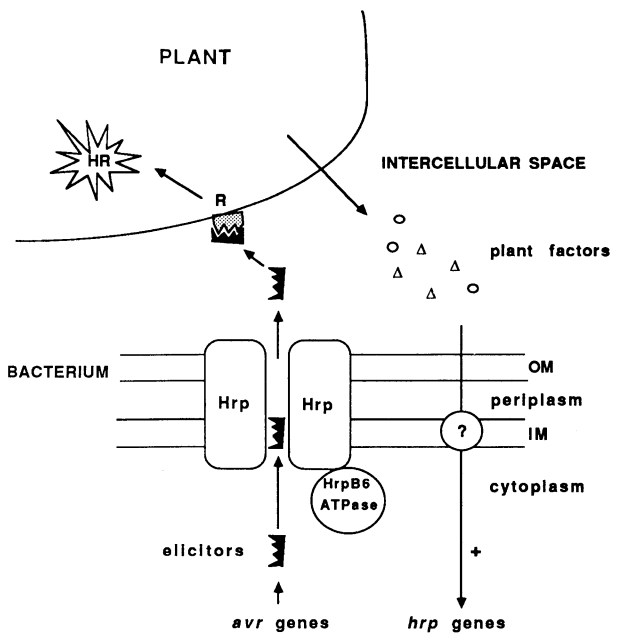

Fig. 6. Hypothetical model of cell signaling between *Xanthomonas campestris* pv. *vesicatoria* and pepper indicating the proposed function of Hrp proteins as a secretion apparatus. *hrp* Genes are regulated by unknown plant factors (Schulte and Bonas 1992). HrpA1, HrpB3, HrpC2, and possibly other Hrp proteins may form a complex (tunnel/pore) that enables the export of molecules such as virulence or avirulence factors (elicitors). "R" refers to a plant receptor (e.g., the product of a resistance gene). In contrast to the situation leading to the hypersensitive response (HR) in resistant plants as depicted here, secretion of so far unknown bacterial virulence factors leads to disease in susceptible plants.

Our findings provide new insights into the general concept of bacterial pathogenicity. We predict that similar functions will be found for Hrp proteins in other bacterial plant pathogens besides *P. solanacearum* (Gough *et al.* 1992). The highly conserved nature of the proteins from plant and animal pathogens raises questions about the evolution of parasitism. Do the sequences described here share a common origin in evolution or did intergeneric gene transfer occur? The fact that some of the Hrp proteins are similar to proteins in *Bacillus*, *Caulobacter*, *Salmonella*, *Shigella*, and *Yersinia* might indicate that general bacterial pathways, such as those involved in secretion, have been adapted by pathogenic organisms to function as part of their pathogenicity mechanism.

ACKNOWLEDGMENTS

We thank Martina Gutschow for technical assistance and Julian Adams, Michael Bölker, Regine Hakenbeck, Torsten Horns, Regine Kahmann, John Mansfield, and Ralf Schulte for helpful suggestions and critical reading of the manuscript. We also thank Jürgen Heesemann for discussion and Christian Boucher and his colleagues for communicating sequence information in advance of publication. This research was supported in part by grants from the Bundesministerium für Forschung und Technologie (322-4003-0316300A) and the EEC (BIOT-CT90-0168) to U.B.

LITERATURE CITED

Albertini, A. M., Caramori, T., Crabb, W. D., Scoffone, F., and Galizzi, A. 1991. The *flaA* locus of *Bacillus subtilis* is part of a large operon coding for flagellar structures, motility functions, and an ATPase-like polypeptide. J. Bacteriol. 173:3573-3579.

Arlat, M., Gough, C. L., Barber, C. E., Boucher, C., and Daniels, M. J. 1991. *Xanthomonas campestris* contains a cluster of *hrp* genes related to the larger *hrp* cluster of *Pseudomonas solanacearum*. Mol. Plant-Microbe Interact. 4:593-601.

Arlat, M., Gough, C. L., Zischek, C., Barberis, P. A., Trigalet, A., and Boucher, C. 1992. Transcriptional organization and expression of the large *hrp* gene cluster of *Pseudomonas solanacearum*. Mol. Plant-Microbe Interact. 5:187-193.

Beaulieu, C., Minsavage, G. V., Canteros, B. I., and Stall, R. E. 1991. Biochemical and genetic analysis of a pectate lyase gene from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Plant-Microbe Interact. 4:446-451.

Bonas, U., Schulte, R., Fenselau, S., Minsavage, G. V., Staskawicz, B. J., and Stall, R. E. 1991. Isolation of a gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and the hypersensitive response on pepper and tomato. Mol. Plant-Microbe Interact. 4:81-88.

Bonas, U., Stall, R. E., and Staskawicz, B. J. 1989. Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. 218:127-136.

Boucher, C., Van 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.

Cornelis, G. R., Biot, T., Lambert de Rouvroit, C., Michiels, T., Mulder, B., Sluiters, C., Sory, M.-P., Van Bouchaute, M., and Vanooteghem, J.-C. 1989. The *Yersinia yop* regulon. Mol. Microbiol. 3:1455-1469.

Daniels, M. J., Dow, J. M., and Osbourn, A. E. 1988. Molecular genetics of plant pathogenic bacteria. Annu. Rev. Phytopathol. 26:285-312.

Delepelaire, P., and Wandersman, C. 1989. Protease secretion by *Erwinia chrysanthemi*: Proteases B and C are synthesized and secreted as zymogens without a signal peptide. J. Biol. Chem. 264:9083-9089.

Devereux, J., Haeberli, P., and Smithson, O. 1984. A comprehensive set of sequence analysis programmes for the VAX. Nucleic Acids Res. 12:387-395.

Dums, F., Dow, J. M., and Daniels, M. J. 1991. Structural characterization of protein secretion genes of the bacterial phytopathogen *Xanthomonas*

campestris pathovar *campestris*: Relatedness to secretion systems of other gram-negative bacteria. Mol. Gen. Genet. 229:357-364.

d'Enfert, C., Reyss, I., Wandersman, C., and Pugsley, A. P. 1989. Protein secretion by gram-negative bacteria. J. Biol. Chem. 264:17462-17468.

Felmlee, T., Pellett, S., Lee, E.-Y., and Welch, R. A. 1985. *Escherichia coli* hemolysin is released extracellularly without cleavage of a signal peptide. J. Bacteriol. 163:88-93.

Fellay, R., Rahme, L. G., Mindrinos, M. N., Frederick, R. D., Pisi, A., and Panopoulos, N. J. 1991. Genes and signals controlling the *Pseudomonas syringae* pv. *phaseolicola*-plant interaction. Pages 45-52 in: Advances in Molecular Genetics of Plant Microbe-Interactions. Vol. 1. H. Hennecke and D. P. S. Verma, eds. Kluwer Academic Publishers, Netherlands.

Finlay, B. B., and Falkow, S. 1989. Common themes in microbial pathogenicity. Microbiol. Rev. 53:210-230.

Goguen, J. D., Yother, J., and Straley, S. C. 1984. Genetic analysis of the low calcium response in *Yersinia pestis* Mu d1(Ap *lac*) insertion mutants. J. Bacteriol. 160:842-848.

Gough, C. L., Genin, S., Zischek, C., and Boucher, C. A. 1992. *hrp* Genes of *Pseudomonas solanacearum* are homologous to pathogenicity determinants of animal pathogenic bacteria and are conserved among plant pathogenic bacteria. Mol. Plant-Microbe Interact. 5:384-389.

Grimm, C., and Panopoulos, N. J. 1989. The predicted protein product of a pathogenicity locus from *Pseudomonas syringae* pv. *phaseolicola* is homologous to a highly conserved domain of several procaryotic regulatory proteins. J. Bacteriol. 171:5031-5038.

Hale, T. L. 1991. Genetic basis of virulence in *Shigella* species. Microbiol. Rev. 55:206-224.

Heesemann, J., Gross, U., Schmidt, N., and Laufs, R. 1986. Immunochemical analysis of plasmid-encoded proteins released by enteropathogenic *Yersinia* sp. grown in calcium-deficient media. Infect. Immun. 54:561-567.

Higgins, C. F., Hiles, I. D., Salmond, G. P., Gill, D. R., Downie, J. A., Evans, I. J., Holland, I. B., Gray, L., Buckel, S. D., Bell, A. W., and Hermodson, M. A. 1986. A family of related ATP-binding subunits coupled to many distinct biological processes in bacteria. Nature (London) 323:448-450.

Hillen, W., and Schollmeier, K. 1983. Nucleotide sequence of the Tn*10* encoded tetracycline resistance gene. Nucleic Acids Res. 11:525-539.

Hu, N.-T., Hung, M.-N., Chiou, S.-J., Tang, F., Chiang, D.-C., Huang, H.-Y., and Wu, C.-Y. 1992. Cloning and characterization of a gene required for the secretion of extracellular enzymes across the outer membrane by *Xanthomonas campestris* pv. *campestris*. J. Bacteriol. 174:2679-2687.

Kamoun, S., and Kado, C. I. 1990. A plant-inducible gene of *Xanthomonas campestris* pv. *campestris* encodes an exocellular component required for growth in the host and hypersensitivity on nonhosts. J. Bacteriol. 172:5165-5172.

Knoop, V., Staskawicz, B. J., and Bonas, U. 1991. The expression of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria* is not under the control of *hrp* genes and is independent of plant factors. J. Bacteriol. 173:7142-7150.

Lindgren, P. B., Frederick, R., Govindarajan, A. G., Panopoulos, N. J., Staskawicz, B. J., and Lindow, S. E. 1989. An ice nucleation reporter gene system: Identification of inducible pathogenicity genes in *Pseudomonas syringae* pv. *phaseolicola*. EMBO J. 5:1291-1301.

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

Michiels, T., and Cornelis, G. R. 1990. Secretion of Yop proteins by the Yersiniae. Infect. Immun. 58:2840-2849.

Michiels, T., and Cornelis, G. R. 1991. Secretion of hybrid proteins by the *Yersinia* Yop export system. J. Bacteriol. 173:1677-1685.

Michiels, T., Vanooteghem, J.-C., Lambert de Rouvroit, C., China, B., Gustin, A., Boudry, P., and Cornelis, G. R. 1991. Analysis of *virC*, an operon involved in the secretion of Yop proteins by *Yersinia enterocolitica*. J. Bacteriol. 173:4994-5009.

Plano, G. V., Barve, S. S., and Straley, S. C. 1991. LcrD, a membrane-bound regulator of the *Yersinia pestis* low-calcium response. J. Bacteriol. 173:7293-7303.

Rahme, L. G., Mindrinos, M. N., and Panopoulos, N. J. 1991. Genetic and transcriptional organization of the *hrp* cluster of *Pseudomonas syringae* pv. *phaseolicola*. J. Bacteriol. 173:575-586.

Rahme, L. G., Mindrinos, M. N., and Panopoulos, N. J. 1992. Plant and environmental sensory signals control the expression of *hrp* genes in *Pseudomonas syringae* pv. *phaseolicola*. J. Bacteriol. 174:3499-3507.

Ramakrishnan, G., Zhao, J.-L., and Newton, A. 1991. The cell cycle-regulated flagellar gene *flbF* of *Caulobacter crescentus* is homologous to a virulence locus (*lcrD*) of *Yersinia pestis*. J. Bacteriol. 173:7283-7392.

Sanders, L. A., Van Way, S., and Mullin, D. A. 1992. Characterization of the *Caulobacter crescentus flbF* promoter and identification of the inferred FlbF product as a homolog of the LcrD protein from a *Yersinia enterocolitica* virulence plasmid. J. Bacteriol. 174:857-866.

Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74:5463-5467.

Saraste, M., Gay, N. J., Eberle, A., Runswick, M. J., and Walker, J. E. 1981. The *atp* operon: Nucleotide sequences of the genes for the α, β, and γ subunits of *Escherichia coli* ATP synthase. Nucleic Acids Res. 9:5287-5296.

Schulte, R., and Bonas, U. 1992. The expression of the *hrp* gene cluster from *Xanthomonas campestris* pv. *vesicatoria* that determines pathogenicity and hypersensitivity on pepper and tomato is plant-inducible. J. Bacteriol. 174:815-823.

Venkatesan, M. M., Buysse, J. M., and Oaks, E. V. 1992. Surface presentation of *Shigella flexneri* invasion plasmid antigemns requires the products of the *spa* locus. J. Bacteriol. 174:1990-2001.

Viitanen, A.-M., Toivanen, P., and Skurnik, M. 1990. The *lcrE* gene is part of an operon in the *lcr* region of *Yersinia enterocolitica* O:3. J. Bacteriol. 172:3152-3162.

Vogler, A. P., Homma, M., Irikura, V. M., and Macnab, R. M. 1991. *Salmonella typhimurium* mutants defective in flagellar filament regrowth and sequence similarity of FliI to F_0F_1, vacuolar, and archaebacterial ATPase subunits. J. Bacteriol. 173:3564-3572.

Walker, J. E., Saraste, M., Runswick, M. J., and Gay, N. J. 1982. Distantly related sequences in the α- and β-subunits of ATP synthase, myosin, kinases and other ATP-requiring enzymes and a common nucleotide binding fold. EMBO J. 1:945-951.

Wei, Z.-M., Sneath, B. J., and Beer, S. V. 1992. Expression of *Erwinia amylovora hrp* genes in response to environmental stimuli. J. Bacteriol. 174:1875-1882.

Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. *hrp* Genes of phytopathogenic bacteria. Mol. Plant-Microbe Interact. 4:132-138.

Wu, H. C., and Tokunaga, M. 1986. Biogenesis of lipoproteins in bacteria. Curr. Top. Microbiol. Immunol. 125:127-157.

Xiao, Y., Lu, Y., Heu, S., and Hutcheson, S. W. 1992. Organizational and environmental regulation of the *Pseudomonas syringae* pv. *syringae* 61 *hrp* cluster. J. Bacteriol. 174:1734-1741.

Yoshida, M., Allison, W. S., Esch, F. S., and Futai, M. 1982. The specificity of carboxyl group modification during the inactivation of the *Escherichia coli* F1-ATPase with dicyclohexyl [^{14}C] carbodiimide. J. Biol. Chem. 257:10033-10037.