Research Note

The Presence of *hrp* Genes on the Pathogenicity-Associated Plasmid of the Tumorigenic Bacterium *Erwinia herbicola* pv. *gypsophila*

Roni Nizan,¹ Isaac Barash,¹ Lea Valinsky,^{1,2} Amnon Lichter,¹ and Shulamit Manulis²

¹Department of Plant Sciences, Tel-Aviv University, Tel-Aviv 69978; and ²Department of Plant Pathology, ARO, The Volcani Center, P.O.B. 6, Bet Dagan 50250, Israel.

Received 4 November 1996. Accepted 21 March 1997.

The pathogenicity-associated plasmid (pPATH) of *Erwinia herbicola* pv. *gypsophila* (*Ehg*), which is present only in pathogenic strains, contains a gene cluster encoding indole-3-acetic acid and cytokinin biosynthesis. The transposon-reporter Tn3-Spice was used to generate nonpathogenic mutants on two overlapping cosmids, pLA150 and pLA352, of the pPATH. A cluster of such mutations, which spanned 16 kb, mapped approximately 15 kb from the gene cluster involved in phytohormone biosynthesis. Nonpathogenic mutants also failed to elicit the hypersensitive reaction (HR) on tobacco. Pathogenicity and HR were restored concomitantly to these mutants by in trans complementation with wild-type *Ehg* DNA. A 3.8-kb *Hind*III DNA fragment that complemented the *hrp* mutants was sequenced and six complete and two partial open reading frames (ORFs) were identified. Comparison of the deduced amino acid sequences of the eight ORFs showed striking homology and co-linearity with *hrp* genes of *E. amylovora* as well as with other plant and mammalian pathogenic bacterial genes encoding proteins of the type III secretion system. Limited DNA sequencing at various sites on the remaining 11-kb region of pLA352 also showed high identity to Hrp proteins of *E. amylovora*, *E. stewartii*, and *Pseudomonas syringae*. These results suggest that *hrp* genes are mandatory for gall formation by *E. herbicola* pv. *gypsophila*.

Additional keywords: phytopathogenic bacteria.

Erwinia herbicola is widespread in nature as an epiphyte on many plants (Starr 1981). However, some strains of *E. herbicola* have evolved into plant pathogens inciting gall formation in gypsophila (*Gypsophila paniculata* L.) (Cooksey 1986) and table beet (*Beta vulgaris* L.) (Burr et al. 1991). The host range of *E. herbicola* pv. *gypsophila* (*Ehg*) is apparently restricted to gypsophila (Volcani 1985), whereas the beet pathovar generates galls on table beet as well as gypsophila (Burr et al.

1991). The pathogenicity of both pathovars is associated with a similar indigenous plasmid (pPATH) of approximately 150 kb that is present exclusively in all pathogenic strains (Manulis et al. 1991; S. Manulis and I. Barash, *unpublished*).

Previous studies have shown that *Ehg* possesses the genes for the production of the phytohormones indole-3-acetic acid (IAA) and cytokinins. Two major metabolic pathways for IAA biosynthesis were characterized. The indole-3-pyruvate (IPVA) route was present on the chromosome of both pathogenic and nonpathogenic strains, whereas the indole-3-acetamide (IAM) pathway was present only in pathogenic strains and was located on the pPATH (Manulis et al. 1991; Clark et al. 1993). Nucleotide sequencing of the cytokinin biosynthetic gene (*etz*) (Lichter et al. 1995a, 1995b) revealed that *etz* was preceded by another open reading frame (ORF) (pre-*etz*), and that the two genes constitute an operon (Lichter et al. 1995b). The genes of the IAM pathway for IAA biosynthesis and the cytokinin genes form a cluster on the plasmid pPATH. The contribution of IAA and cytokinins to gall formation was assessed by gene disruption with both transposon insertions and deletion mutagenesis. Inactivation of either IAA production through the IAM pathway (Clark et al. 1993) or cytokinin biosynthesis (Lichter et al. 1995a), as well as simultaneous inactivation of the two IAA pathways and cytokinin biosynthesis (Haviv-Chesner 1996), caused a substantial reduction in gall size but did not eliminate gall initiation. These results may suggest that gall initiation is triggered by genes other than those producing phytohormones.

The presence of *hrp* genes is essential for pathogenicity in most gram-negative bacteria (Bonas 1994). Mutations in *hrp* genes abolish pathogenicity in susceptible host plants and abolish the hypersensitive reaction (HR) in resistant host and nonhost plants. Extensive genetic studies in various bacterial wild-type strains of *Erwinia*, *Pseudomonas*, *Xanthomonas*, and *Ralstonia* spp. have demonstrated that *hrp* genes are organized in clusters of 22 to 40 kb and contain at least six to eight complementation groups (Willis et al. 1991; Bonas 1994). The nucleotide sequences of *hrp* gene clusters revealed high similarities between pathogens belonging to different genera and some regions appear to be highly conserved (e.g., Gough et al. 1992; Huang et al. 1995; Fenselau and Bonas 1995; Bogdanove et al. 1996a). A main component of the *hrp*

Corresponding authors: I. Barash, E-mail: ISAACI@post.tau.ac.il; S. Manulis, E-mail: VPSHULA@volcani.agri.gov.il

Nucleotide and/or amino acid sequence data can be found at GenBank as accession no. X99768.

cluster is a novel and elaborate protein secretion system termed type III (Salmond and Reeves 1993). Proteins secreted by this pathway lack typical signal sequences, are not processed during transfer, and do not have a periplasmic intermediate stage. They can be transferred directly into the host cell by a process defined as polarized translocation (Rosqvist et al. 1994). The type III system mediates the secretion of virulence factors produced by gram-negative mammalian pathogenic bacteria such as *Yersinia*, *Shigella*, and *Salmonella* spp., as well as export of proteins needed for flagellar biogenesis (Van Gijsegem et al. 1993; Cornelis 1994; Parsot 1994; Finlay 1994). Recently the type III system has been demonstrated to mediate the secretion of harpin from plant-pathogenic bacteria and appears to be involved in the transfer of *avr* products into plant cells (Bogdanove et al. 1996b; Pirhonen et al. 1996; Gopalan et al. 1996). Regulatory genes constitute an additional component of the *hrp* cluster allowing the bacteria to sense the host environment. The *hrp* genes in various *P. syringae* pathovars, for example, are positively regulated by HrpS and HrpR, which are members of the NtrC family of regulatory proteins (Grimm et al. 1995). These proteins are responsible for *hrpL* activation, which in turn initiates transcription of the various *hrp* operons (Xiao et al. 1994). The *hrp* clusters also are characterized by the presence of a gene encoding for harpin that triggers HR response in nonhost plants (Bonas 1994). The function of other *hrp* genes is still unknown.

It is noteworthy that all the *hrp* gene clusters have been reported to be localized on the chromosome (Willis et al. 1991) with the exception of *R. solanacearum*, which harbors the *hrp* cluster on a megaplasmid (Boucher et al. 1987). Furthermore, there seem to be no *hrp* gene equivalents present in *Agrobacterium tumefaciens* (Laby and Beer 1992) nor have there been any reports of *hrp* genes in any other tumorigenic bacteria. In this preliminary report we demonstrate that the pPATH of *Ehg* contains pathogenicity genes that exhibit the *hrp* phenotype and possess high homology with genes of the type III secretion system and possibly other *hrp* genes.

The bacterial strains, cosmids, and plasmid constructs used in this study are presented in Table 1. Bacteria were grown in Luria-Bertani (LB) broth or on LB agar plates at 28°C for *Ehg* and 37°C for *Escherichia coli*. Mutagenesis was carried out on

two cosmid clones of the pPATH, pLA150 and pLA352, which were obtained from a plasmid library of Eh824-1 in pLAFR3 (Lichter et al. 1995b). Insertional mutagenesis was performed with the transposon reporter pTn3-Spice as described by Lindgren et al. (1989). The mutated cosmids were mobilized to the wild-type strain Eh824-1 by triparental mating with pRK2073 as a helper plasmid (Ditta et al. 1980). Plasmid curing was performed as previously described (Lichter et al. 1995b). The marker exchange mutants were subjected to pathogenicity tests on cuttings of *Gypsophila paniculata* var. *perfecta* essentially as described previously (Manulis et al. 1991). After removal of a 2- to 3-mm section from the bottom of the stem, the cutting was inoculated by dipping into a bacterial suspension of 10⁸ CFU/ml and placed in trays filled with vermiculite. Greenhouse temperature was maintained at 26 to 29°C and high humidity was generated by computer-controlled mist sprinklers (Lichter et al. 1995a). Gall formation was scored 14 days after inoculation on 3 or 4 replicas per strain in at least two independent experiments. For hypersensitivity tests, bacteria were grown in an inducing medium (pH 5.5; modified from D. Coplin, *personal communication*) composed of the following compounds: (NH₄)₂SO₄, 1 mM; KH₂PO₄, 0.5 mM; MgSO₄, 0.5 mM; morpholine-ethanesulfonic acid (MES) buffer, 50 mM; casamino acids, 0.05%; sucrose, 2%. HR tests were carried out by infiltrating bacterial cell suspension (10⁹ CFU/ml) into the mesophyll of tobacco leaves (*Nicotiana tabacum* L. 'Hicks') as described elsewhere (Bauer and Beer 1991). HR was recorded after 48 h.

Hrp⁻ mutants that were nonpathogenic on gypsophila and failed to elicit HR on tobacco were obtained by Tn3-Spice insertions. The location and orientation of these mutants were determined by mapping with multiple restriction enzymes and hybridization with a 1.5-kb *Bam*HI DNA fragment derived from Tn3-Spice as a probe. The leftward insertion mutant on pLA352 mapped approximately 15 kb to the right of the IAA-cytokinin gene cluster, and the rest of the Tn3-Spice insertions spanned along 16 kb up to the right on this cosmid (Fig. 1). Pathogenicity was restored to all of the Hrp⁻ marker exchange mutants following mobilization of pLA352 into each of the mutant strains by triparental matings. In addition, comple-

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

Strain/plasmid	Relevant characteristics ^a	Reference source
<i>Escherichia coli</i> HB101	F ⁻ <i>hsdS20 (hsdR hsdM) recA13 thi leu proA2 lacY1 galK2 rpsL xgl-5 met-1 supE44</i>	
<i>Erwinia herbicola</i> pv. <i>gypsophila</i> Eh824-1	Wild-type pathogenic strain Rif ^r serotype I	Manulis et al. 1991
Eh824-1Mx(#)	Marker exchange mutants on the pathogenicity-associated plasmid (pPATH)	This study
Cosmids		
pLAFR3	Tc ^r , broad host range vector IncP-1 <i>rlxRK2⁺ lacZa Tra⁻ Mob⁺ cos</i>	Staskawicz et al. 1987
pLA150	Tc ^r , a 28-kb cosmid of pPATH in pLAFR3	Lichter et al. 1995b
pLA352	Tc ^r , a 23.5-kb cosmid of pPATH in pLAFR3	This study
pLA-H3.8	Tc ^r , a 3.8-kb <i>Hind</i> III fragment from pLA352 in pLAFR3	This study
Plasmids		
pBluescript	Amp ^r , subcloning and sequencing vector, (pBS)	Stratagene (La Jolla, CA)
pRK2073	Spec ^r , Strep ^r , helper plasmid for triparental matings	Ditta et al. 1980
pTn3-Spice	Amp ^r , Spec ^r , clone containing the <i>inaZ</i> reporter gene in Tn3	Lindgren et al. 1989
pMBN-H3.8	Amp ^r , a 3.8-kb <i>Hind</i> III fragment from pLA352 in pBluescript	This study

^a Antibiotics were used in the following concentrations (µg per ml): ampicillin (Amp) 100; kanamycin (Km) 50; spectinomycin (Spec) 50; rifampicin (Rif) 100; naladixic acid (Nal) 50; and tetracycline (Tc) 12.

mentation in trans of the Hrp⁻ mutants located on the 3.8-kb *Hind*III fragment of pLA352, namely, Eh824-1M(x) (# = 1,4,5,12,18,19,28) also was achieved by pLA-H3.8, the non-mutated homolog. The marker exchange mutants located on the 3.8-kb *Hind*III fragment of pLA352 (Fig. 1) were tested for HR elicitation and failed to cause HR on tobacco. These mutants restored HR response by complementation in trans with pLA-H3.8. The former and latter results indicate that the mutants were phenotypically Hrp, i.e., they were unable to produce either gall formation or HR on host and nonhost plants, respectively. The 3.8-kb *Hind*III fragment (pMBN-H3.8) was entirely sequenced in two directions by the dideoxy chain termination method with the Sequenase 2.0 kit (US Biochemical, Cleveland, OH) and $\alpha^{35}\text{S}$ -dATP (Du Pont, NEN, Boston, MA) as previously described (Lichter et al. 1995b). Generation of unidirectional deletions of the 3.8-kb *Hind*III fragment for sequencing was obtained with the "Erase a Base" system (Promega, Madison, WI). The nucleotide sequence of pMBN-H3.8 has been submitted to the EMBL, GenBank, and DDJB data bases as accession number X99768. Further limited sequencing was carried out at different sites on pLA352 as indicated in Figure 1. This was done by generating sub-clones in pBluescript (Stratagene, La Jolla, CA) and sequencing their termini by automated sequencing with *Taq* DNA polymerase (Department for Biological Services, Weizmann Institute, Rehovot, Israel). Analysis of DNA and deduced protein sequences was performed with the Program Manual for the Wisconsin Package (Version 8, Sept. 1994, Genetic Computer Group, Madison, WI).

The DNA sequence of the 3.8-kb *Hind*III fragment revealed six complete (numbered 2 to 7) and two partial (numbered 1 and 8) adjacent ORFs (Fig. 1). The translational direction of

the ORFs also was in agreement with the ice nucleation reporter gene (*inaZ*) in the Tn3-Spice (Nizan-Koren 1995). Comparison of the deduced amino acid sequences of the eight ORFs to sequences in the data base showed striking homology to genes of *E. amylovora* encoding proteins of the type III secretion system (Table 2). In addition to the remarkable conservation of this gene cluster, the gene order was also maintained between the two species. However, in contrast to *E. amylovora* (Bogdanove et al. 1996b) and similar to HrcQ_A of *P. syringae* pv. *syringae* (Huang et al. 1995; Bogdanove et al. 1996a), the predicted product of ORF3 was aligned with the N-terminal two-thirds of HrcQ of *E. amylovora*, whereas ORF4 was aligned with the third C-terminal sequence of HrcQ, as was HrcQ_B of *P. syringae* pv. *syringae* (Table 2). Consequently, ORF3 and ORF4 of *Ehg* were named *hrcQ_A* and *hrcQ_B*, respectively. GTG rather than ATG is presumably the start codon of ORF2, compared with HrpP of *E. amylovora* (Bogdanove et al. 1996b). Sample sequences taken from various sites on the remaining region of pLA352 (Fig. 1) also showed high homology with Hrp proteins of *E. amylovora*, *E. stewartii*, and *P. syringae* pv. *syringae* (R. Nizan, S. Manulis, and I. Barash, *unpublished*). Thus, samples 1, 2, and 3 showed identity of 70.5% (per 280 amino acids [aa]), 73.4% (per 280 aa), and 57% (per 183 aa), respectively, with HrcN, HrcV, and HrpL of *E. amylovora* (Bogdanove et al. 1996b; Wei and Beer 1995). Samples 1 and 2 also showed lower identity to corresponding genes in *P. syringae* pv. *syringae* and *X. campestris* (Hwang et al. 1992; Huang et al. 1995). Sample 4 showed identity of 65.4% (per 240 aa) with HrpS of *E. stewartii* and lower identity with HrpS of *P. syringae* pv. *phaseolicola* or HrpR of *P. syringae* pv. *syringae* (Frederick et al. 1993; Grimm et al. 1995; Huang et al. 1995). Sample 6 exhibited

Fig. 1. Partial physical map of cosmid clones pLA150 and pLA352 of the pathogenicity-associated plasmid (pPATH). Open reading frames (ORFs) in pMBN-H3.8 indicated by open boxes. Arrow signifies transcriptional orientation. Sites of sample sequences on pLA352 indicated by open ellipsoids with the corresponding deduced Hrp proteins to which sequence identity was detected (see text). Marker exchange mutants, indicated by black triangles, are pointed toward the transcription orientation of the ice nucleation gene (*inaZ*) in the transposon-reporter Tn3-Spice. *iaaM* and *iaaH* are 3-indoleacetic acid (IAA) biosynthetic genes, whereas *pre-etz* and *etz* constitute the cytokinin biosynthetic operon. Abbreviations: B, *Bam*HI; H, *Hind*III; R, *Eco*RI.

71% identity (per 186 aa) with HrcJ of *E. amylovora*, and lower identity with *P. syringae* pv. *syringae* and *X. campestris*. No homology could be detected for sample 5 in the data base.

A unified *hrp* nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria has recently been suggested by Bogdanove et al. (1996a) and was adopted in this study. In the present work we have either fully or partially identified the following deduced proteins of the type III system in *Ehg*: HrcJ, HrcV, HrcN, HrpO, HrpP, HrcQ_A, HrcQ_B, HrcR, HrcS, HrcT, and HrcU (Table 2; R. Nizan, S. Manulis, and I. Barash, *unpublished results*). Nine of these genes are homologous to the broadly conserved genes of the type III system that were designated as *hrc* (Bogdanove et al. 1996a). It is noteworthy that eight of the identified genes (i.e., *hrcN* to *hrcU*) are co-linear with their counterparts in the *lcrB(yscN-U)* gene cluster of *Y. pseudotuberculosis* that are involved in Yop secretion. Some of these genes (e.g., *hrcQ_B*, *hrcR*, *hrcS*, and *hrcT*) show high homology to *yscQ-T* of *Yersinia* and *spa* genes of *Salmonella* and *Shigella* (Table 2) (Bergman et al. 1994). *hrcV* of *Ehg*, formerly *hrpI* of *E. amylovora* and *P. syringae*, showed exceptionally high homology (75% identity per 229 aa) to *P. syringae* (R. Nizan, S. Manulis, and I. Barash, *unpublished results*). Based on sequence analysis, the *hrcV-U* genes with the preceding gene *hrpJ* (not yet detected in *Ehg*) are considered to constitute a large operon in *E. amylovora* (Bogdanove et al. 1996b). However, this DNA spans previously defined complementation groups VI, VII, and VIII of this bacterium. Therefore, further genetic and biochemical analysis is required to resolve the transcriptional organization of this region in *E. amylovora* as well as in *Ehg*.

The detection of sequences related to HrpL of *E. amylovora* and HrpS of *E. stewartii* on pLA352 (samples 3 and 4 in Figure 1) suggests that this DNA region may contain *hrp* regulatory genes. *hrpL* is a positive regulatory gene that controls the

transcription of other *hrp* genes, including genes of the type III system that are required for harpin secretion (Wei and Beer 1995). *hrpL* alone was sufficient to activate *hrp* promoters in *P. syringae* as well as in *avr* genes and it may function as an alternate sigma factor (Xiao et al. 1994; Salmeron and Staskiewicz 1993). HrpS was previously identified as a transcriptional regulator (Frederick et al. 1993).

The results of this study clearly suggest the presence of *hrp* genes on the pPATH and their involvement in gall formation. *hrp* genes have so far been demonstrated only in necrogenic, gram-negative, phytopathogenic bacteria (Bonas 1994). This report indicates that they also may be present and function in tumorigenic bacteria, although not in *A. tumefaciens* (Laby and Beer 1992). Thus, our findings that a gall-forming bacterium does contain functional *hrp* genes demonstrate a fundamental difference between *E. herbicola* and *A. tumefaciens*. It has previously been shown that, in contrast to the phytohormone-independent growth pattern exhibited in axenic culture by *A. tumefaciens*-transformed gypsophila tumor, galls caused by *Ehg* are dependent on external cytokinins and auxin (Clark et al. 1989). The latter results imply that *Ehg* does not transform phytohormone genes into gypsophila, unlike *A. tumefaciens*. *A. tumefaciens* possesses a unique mechanism for DNA-mediated tumorigenic transformation of the host plant, employing an elaborate set of genes specific for that purpose (Zambryski 1992). In contrast, *E. herbicola* has to rely on phytohormones produced by the pathogen and as yet unknown virulence proteinaceous factors secreted by the *hrp* system.

The absolute correlation between the presence of pPATH and pathogenicity of *Ehg* (Manulis et al. 1991), as well as the numerous nonpathogenic mutants already demonstrated on this plasmid (this work and I. Barash and S. Manulis, *unpublished*), lead us to hypothesize that most if not all of the genes necessary for the conversion of *Ehg* into a pathogen have been assembled

Table 2. Similarities between putative protein coding sequences identified in the 3.8-kb *HindIII* fragments of pathogenicity-associated plasmid (pPATH) and deduced protein sequences from other bacteria

Bacteria	ORF1 ^a 131 aa	ORF2 156 aa	ORF3 227 aa	ORF4 109 aa	ORF5 217 aa	ORF6 76 aa	ORF7 265 aa	ORF8 ^b 52 aa	Reference
<i>E. amylovora</i>	HrpO 60.8% ^c	HrpP 64.3%	HrcQ ^d 56.4%	HrcQ ^e 73.4%	HrcR 90.4%	HrcS 96.0%	HrcT 81.5%	HrcU 94.4%	Bogdanove et al. 1996b
<i>P. syringae</i>			HrcQA 29.7%	HrcQB 40.4%	HrcR 69.4%	HrcS 69.4%	HrcT 47.3%	HrcU 55.6%	Huang et al. 1995
<i>R. solanacearum</i>					HrcR 36.4%	HrcS 50.0%	HrcT 28.1%		Van Gijsegem et al. 1995
<i>X. campestris</i>					ORF2 40.0%				Hwang et al. 1992;
<i>Yersinia</i> spp.				YscQ ^f 28.8%	YscR 42.2%	YscS 46.7%	YscT 31.1%		Bergman et al. 1994
<i>S. typhimurium</i>				SpaO ^g 27.6%	SpaP 33.0%	SpaQ 46.7%	SpaR 25.6%		Grolsman and Ochman 1993
<i>S. flexneri</i>					Spa24 34.1%		Spa29 27.2%		Sasakawa et al. 1993
<i>B. subtilis</i>					FliP 34.6%	FliQ 33.8%	FliR 23.4%		Bischoff et al. 1992; Carpenter and Ordal 1993
<i>E. carotovora</i>					MopC 36.5%	MopD 35.5%	MopE 23.6%		Mulholland et al. 1993

^a Partial open reading frame (ORF) (C-terminal sequence). aa = amino acid.

^b Partial ORF (N-terminal sequence).

^c Percent identity deduced amino acids.

^d The whole sequence was aligned to a two third N-terminal sequence of HrcQ.

^e The whole sequence was aligned to a third C-terminal sequence of HrcQ.

^f The whole sequence was aligned to a fourth C-terminal sequence of YscQ.

^g The whole sequence was aligned to a fourth C-terminal sequence of SpaO.

on the pPATH. Since insertional mutagenesis in genes corresponding to the type III system completely abolished pathogenicity, it may be concluded that crucial proteins for infection are secreted through this system. The plasmid linkage of these putative *hrp* genes in association with the phytohormone gene cluster (Clark et al. 1993; Lichter et al. 1995b) and the pPATH-specific insertion element *IS1327* (Lichter et al. 1996) may suggest a unique evolutionary route for introducing pathogenicity to *E. herbicola*. Therefore, the pPATH provides a special target of about 150 kb for rigorous analysis and isolation of genes encoding virulence proteins mediated by the type III system. Such genes may be physically linked to the *hrp* cluster or located in other regions of the pPATH.

ACKNOWLEDGMENTS

This study was supported by grant US-2505-94 from the US-Israel Binational Agricultural Research and Development Fund (BARD).

LITERATURE CITED

- Bauer, D. W., and Beer, S. V. 1991. Further characterization of an *hrp* gene cluster of *Erwinia amylovora*. Mol. Plant-Microbe Interact. 4: 493-499.
- Bergman, T., Erickson, K., Galyov, E., Persson, C., and Wolf-Watz, H. 1994. The *lcrB* (*yscN/U*) gene cluster of *Yersinia pseudotuberculosis* is involved in Yop secretion and shows high homology to the *spa* gene clusters of *Shigella flexneri* and *Salmonella typhimurium*. J. Bacteriol. 176:2619-2626.
- Bischoff, D. S., Weinreich, M. D., and Ordal, G. W. 1992. Nucleotide sequences of *Bacillus subtilis* flagellar biosynthetic genes *fliP* and *fliQ* and identification of a novel flagellar gene *fliZ*. J. Bacteriol. 174:4017-4025.
- Bogdanove, A. J., Beer, S. V., Bonas, U., Boucher, C. A., Collmer, A., Coplin, D. L., Cornelis, G. R., Hunang, H. C., Hutcheson, S. W., Panopoulos, N. J., and Van-Gijsegem, F. 1996a. Unified nomenclature for broadly conserved *hrp* genes of phytopathogenic bacteria. Mol. Microbiol. 20:681-683.
- Bogdanove, A. J., Wei, Z. M., Zhao, L., and Beer, S. V. 1996b. *Erwinia amylovora* secretes harpin via a type III pathway and contains a homolog of *yopN* of *Yersinia*. J. Bacteriol. 178:1720-1730.
- Bonas, U. 1994. *hrp* genes of phytopathogenic bacteria. Curr. Top. Microbiol. Immunol. 192:79-98.
- Boucher, C. A., Van Gijsegem, F., Barberis, P. A., Arlat, M., and Zischek, M. 1987. *Pseudomonas solanacearum* gene controlling both pathogenicity and hypersensitivity on tobacco are clustered. J. Bacteriol. 169:5626-5632.
- Burr, T. J., Katz, B. H., Abawi, G. S., and Crosier, D. C. 1991. Comparison of tumorigenic strains of *Erwinia herbicola* isolated from table beet with *E. h. gypsophylae*. Plant Dis. 75:855-858.
- Carpenter, P. B., and Ordal, G. W. 1993. *Bacillus subtilis* flagellar proteins FliP, FliQ, FliR and FlhB are related to *Shigella flexneri* virulence factors. Gene 137:243-245.
- Clark, E., Manulis, S., Ophir, Y., Barash, I., and Gafni, Y. 1993. Cloning and characterization of *iaaM* and *iaaH* from *Erwinia herbicola* pathovar *gypsophylae*. Phytopathology 83:234-240.
- Clark, E., Vigodsky-Hass, H., and Gafni, Y. 1989. Characteristics in tissue culture of hyperplasias induced by *Erwinia herbicola* pathovar *gypsophylae*. Physiol. Mol. Plant Pathol. 35:383-390.
- Cooksey, D. A. 1986. Galls of *Gypsophila paniculata* caused by *Erwinia herbicola*. Plant Dis. 70:464-468.
- Cornelis, G. R. 1994. *Yersinia* pathogenicity factors. Curr. Top. Microbiol. Immunol. 192:243-263.
- Ditta, G., Stanfield, S., Corbin, D., and Helinsky, D. R. 1980. Broad host range DNA cloning system for gram-negative bacteria: Construction of a gene bank of *Rhizobium meliloti*. Proc. Natl. Acad. Sci. USA 77: 7343-7351.
- Fenselau, S., and Bonas, U. 1995. Sequence and expression analysis of the *hrpB* pathogenicity operon of *Xanthomonas campestris* pv. *vesicatoria* which encodes eight proteins with similarity to components of the Hrp, Ysc, Spa, and Fli secretion systems. Mol. Plant-Microbe Interact. 8:845-854.
- Finlay, B. B. 1994. Molecular and cellular mechanisms of *Salmonella* pathogenesis. Curr. Top. Microbiol. Immunol. 192:163-185.
- Frederick, R. D., Majerczak, D. R., and Coplin, D. L. 1993. *Erwinia stewartii* WtsA, a positive regulator of pathogenicity gene expression, is homologous to *Pseudomonas syringae* pv. *phaseolicola* HrpS. Mol. Microbiol. 9:477-485.
- Gopalan, S., Bauer, D. W., Alfano, J. R., Loniello, A. O., He, S. Y., and Collmer, A. 1996. Expression of the *Pseudomonas syringae* avirulence protein AvrB in plant cells alleviates its dependence on the hypersensitive response and pathogenicity (*Hrp*) secretion system in eliciting genotype-specific hypersensitive cell death. Plant Cell 8:1095-1105.
- 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., Aufsatz, W., and Panopoulos, N. J. 1995. The *hrpRS* locus of *Pseudomonas syringae* pv. *phaseolicola* constitutes a complex regulatory unit. Mol. Microbiol. 15:155-165.
- Grolsman, E. A., and Ochman, H. 1993. Cognate gene clusters govern invasion of host epithelial cell by *Salmonella typhimurium* and *Shigella flexneri*. EMBO J. 12:3779-3787.
- Haviv-Chesner, A. 1996. The effect of inactivation of the genes encoding Auxin and Cytokinin production on the pathogenicity of *Erwinia herbicola* pv. *gypsophylae*. M.Sc. thesis. Tel-Aviv University, Tel-Aviv, Israel.
- Huang, H.-C., Lin, R.-H., Chang, C.-J., Collmer, A., and Deng, W.-L. 1995. The complete *hrp* gene cluster of *Pseudomonas syringae* pv. *syringae* 61 include two blocks of genes required for harpin_{PS} secretion that are arranged colinearly with *Yersinia* *ysc* homologs. Mol. Plant-Microbe Interact. 8:733-746.
- Hwang, I., Lim, S. M., and Shaw, P. D. 1992. Cloning and characterization of pathogenicity genes from *Xanthomonas campestris* pv. *glycines*. J. Bacteriol. 174:1923-1931.
- Laby, R. J., and Beer, S. V. 1992. Hybridization and functional complementation of the *hrp* gene cluster from *Erwinia amylovora* strain Ea321 with DNA of other bacteria. Mol. Plant-Microbe Interact. 5:412-419.
- Lichter, A., Barash, I., Valinsky, L., and Manulis, S. 1995a. The genes involved in cytokinin biosynthesis in *Erwinia herbicola* pv. *gypsophylae*: Characterization and role in gall formation. J. Bacteriol. 177: 4457-4465.
- Lichter, A., Manulis, S., Sagee, O., Gafni, Y., Gray, J., Meilan, R., Morris, R. O., and Barash, I. 1995b. Production of cytokinins by *Erwinia herbicola* pv. *gypsophylae* and isolation of a locus conferring cytokinin biosynthesis. Mol. Plant-Microbe Interact. 8:114-121.
- Lichter, A., Manulis, S., Valinsky, L., Karniol, B., and Barash, I. 1996. *IS1327*, a new insertion-like element in the pathogenicity-associated plasmid of *Erwinia herbicola* pv. *gypsophylae*. Mol. Plant-Microbe Interact. 9:98-104.
- 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. 8:1291-1301.
- Manulis, S., Gafni, Y., Clark, E., Zutra, D., Ophir, Y., and Barash, I. 1991. Identification of a plasmid DNA probe for detection of strains of *Erwinia herbicola* pathogenic on *Gypsophila paniculata*. Phytopathology 81:54-57.
- Mulholland, V., Hinton, J. C. D., Sidebotham, J., Toth, I. K., Hyman, L. J., Perombelon, M. C. M., Reeves, P. J., and Salmond, G. P. C. 1993. A pleiotropic reduced virulence (*Rvi*⁻) mutant of *Erwinia carotovora* subspecies *atroseptica* is defective in flagella assembly proteins that are conserved in plant and animal bacterial pathogens. Mol. Microbiol. 9:343-356.
- Nizan-Koren, R. 1995. Identification of pathogenicity genes on the pPATH plasmid in *Erwinia herbicola*. M.Sc. thesis. Tel-Aviv University, Tel-Aviv, Israel.
- Parsot, C. 1994. *Shigella flexneri*: Genetic of entry and intracellular dissemination in epithelial cells. Curr. Top. Microbiol. Immunol. 192: 217-241.
- Pirhonen, M. U., Lidell, M. C., Rowley, D. L., Lee, S. W., Jin, S., Liang, Y., Silverstone, S., Keen, N. T., and Hutcheson, S. W. 1996. Pheno-

- typic expression of *Pseudomonas syringae* *avr* genes in *E. coli* is linked to the activities of the *hrp*-encoded secretion system. *Mol. Plant-Microbe Interact.* 9:252-260.
- Rosqvist, R., Magnusson, K. E., and Wolf-Watz, H. 1994. Target cell contact triggers expression and polarized transfer of *yersinia* YopE cytotoxin into mammalian cells. *EMBO J.* 13:964-972.
- Salmeron, J. M., and Staskawicz, B. J. 1993. Molecular characterization and *hrp*-dependence of the virulence gene *avrPto* from *Pseudomonas syringae* pv. *tomato*. *Mol. Gen. Genet.* 239:6-10.
- Salmond, G. F. C., and Reeves, J. 1993. Membrane traffic wardens and protein secretion in Gram-negative bacteria. *Trends Biochem. Sci.* 18:7-12.
- Sasakawa, C. K., Komatsu, T., Tobe, T., Suzuki, T., and Yoshikawa, M. 1993. Eight genes in region 5 that form an operon are essential for invasion of epithelial cells by *Shigella flexneri* 2a. *J. Bacteriol.* 175: 5916-5914.
- Starr, M., P. 1981. The genus *Erwinia*. Pages 1260-1271 in: *The Prokaryotes*. M. P. Starr, H. G. Truper, A. Baluws, and H. G. Schlegel, eds. Springer-Verlag, Berlin
- Staskawicz, B., Dhalbeck, D., Keen, N., and Napoli, C. 1987. Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. *J. Bacteriol.* 169:5789-5794.
- Van Gijsegem, F., Genin, S., and Boucher, C. 1993. Conservation of secretion pathways for pathogenicity determinants of plant and animal bacteria. *Trends Microbiol.* 1:175-180.
- Van Gijsegem, F., Gough, C., Zischek, C., Niqueux, E., Arlat, M., Genin, S., Barberis, P., German, S., Castello, P., and Boucher, C. 1995. The *hrp* gene locus of *Pseudomonas solanacearum* which controls the production of type III secretion system encodes eight proteins related to components of the bacterial flagellar biogenesis complex. *Mol. Microbiol.* 15:1095-1114.
- Volcani, Z. 1985. Bacterial diseases of plants in Israel. Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel.
- Wei, Z. B., and Beer, S. V. 1995. *hrpL* activates *Erwinia amylovora* *hrp* gene transcription and is a member of the ECF subfamily of sigma factors. *J. Bacteriol.* 177:6201-6210.
- Willis, D. K., Rich, J. J., and Hrabak, E. M. 1991. *hrp* genes of phytopathogenic bacteria. *Mol. Plant-Microbe Interact.* 4:132-138.
- Xiao, Y., Heu, S., Jinseong, Y., Lu, Y., and Hutcheson, S. W. 1994. Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *P. syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. *J. Bacteriol.* 176: 1025-1036.
- Zambryski, P. C. 1992. Chronicles from the *Agrobacterium*-plant cell DNA transfer story. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43: 465-490.