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**Molecular Basis of the Hrp Pathogenicity of the Fire Blight Pathogen *Erwinia amylovora*: a Type III Protein Secretion System Encoded in a Pathogenicity Island**

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*Erwinia amylovora* causes a devastating disease called fire blight in rosaceous trees and shrubs such as apple, pear, and raspberry. To successfully infect its hosts, the pathogen requires a set of clustered genes termed *hrp*. Studies on the *hrp* system of *E. amylovora* indicated that it consists of three functional classes of genes. Regulation genes including *hrpS*, *hrpXY*, and *hrpL* produce proteins that control the expression of other genes in the cluster. Secretion genes, many of which named *hrc*, encode proteins that may form a transmembrane complex, which is devoted to type III protein secretion. Finally, several genes encode the proteins that are delivered by the protein secretion apparatus. They include harpins, DspE, and other potential effector proteins that may contribute to proliferation of *E. amylovora* inside the hosts. Harpins are glycine-rich heat-stable elicitors of the hypersensitive response, and induce systemic acquired resistance. The pathogenicity protein DspE is homologous and functionally similar to an avirulence protein of *Pseudomonas syringae*. The region encompassing the *hrp/dsp* gene cluster of *E. amylovora* shows features characteristic of a genomic island: a cryptic recombinase/integrase gene and a tRNA gene are present at one end and genes corresponding to those of the *Escherichia coli* K-12 chromosome are found beyond the region. This island, designated the Hrp pathogenicity island, is more than 60 kilobases in size and carries as many as 60 genes.

**Keywords** : HR, Hrp pathway, sigma factor, two-component system, virulence.

**Fire Blight and *Erwinia amylovora***

Fire blight, caused by a bacterial pathogen *Erwinia amylovora*, is one of the most destructive diseases of apples, pears, raspberries, and several ornamental plants (van der Zwet and Beer, 1999; Vanneste, 2000). Affected blossoms, leaves, shoots, fruit, limbs, and, sometimes, entire trees die and become blighted, producing characteristic fire-scorched symptoms. Small droplets of sticky bacterial ooze often can be seen on the surface of blighted tissues. The disease was first observed in the United States, and *E. amylovora* was the first bacterium proven to cause disease in plants. Fire blight is of great economic and political importance worldwide. American strains of *E. amylovora* have spread to many countries in Europe and the Middle East since the turn of the century, and the disease continues to find its way to new countries. During the past several years, outbreaks of fire-blight-like diseases have occurred in Japan, Korea and Australia. Therefore, fire blight poses tremendous problems in international trading of fruits, and it remains as one of the major bacterial pathogens for which plant quarantine measures are in place. Control of the disease is becoming difficult with emergence of antibiotic-resistant strains in many important growing areas.

*E. amylovora* is the type species of its genus and belongs to the family *Enterobacteriaceae* that contains medically important genera such as *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, as well as plant-pathogenic genera such as *Pantoea*, *Pectobacterium*, and *Brenneria*. *E. amylovora* is a highly sophisticated plant pathogen, and, with well-studied *Escherichia coli* and non-plant pathogen *Pantoea agglomerans* (*Erwinia herbicola*) as closely related organisms, it provides a good example for studying the evolution of plant pathogenicity. It is among a handful of phytopatho-

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genic bacteria whose genetic maps were developed in the pre-molecular era. During the 1980s, molecular techniques initially developed for *E. coli* were adapted to the study of *E. amylovora*, and led progress in understanding pathogenicity. For example, transposon mutagenesis revealed several classes of mutants affected in pathogenicity or virulence. Two major factors – *hrp/dsp* genes and genes involved in biogenesis of extracellular polysaccharides – were found to be important in pathogenesis (Kim and Beer, 2000; Bogdanove et al., 2000; Geider, 2000). This paper reviews the discovery of *hrp/dsp* genes in *E. amylovora* and recent developments in our understanding of the roles of these genes in the fire blight pathogenesis.

### Hypersensitive Reaction and *hrp* Genes

Most Gram-negative plant-pathogenic bacteria except for *Agrobacterium* spp. elicit the defensive hypersensitive reaction (HR) in non-host plants instead of causing diseases (Goodman and Novacky, 1994). Macroscopic HR can be easily observed usually 18-24 hr after infiltration of high concentrations of bacterial cells ( $\geq 5 \times 10^6$  cells/ml) into the intercellular spaces of plant leaves. During the HR, rapid  $K^+/H^+$  exchange occurs, and rapidly dying plant cells release (1) toxic compounds that provide a harsh environment for the invading microbes, and (2) signal molecules that alert surrounding cells to induce expression of defense-related genes. Early experiments on the bacteria-elicited HR established that a single bacterium can induce HR of a single plant cell, and that contact between bacteria and plant cells is critical for development of HR.

In the mid-1980s, it became evident that the same factors that control the ability to induce HR in nonhosts control the ability to infect hosts. These genes required for both the HR and pathogenicity, thus called *hrp*, were first identified in *Pseudomonas syringae*, and soon were found in species of *Erwinia*, *Pantoea*, *Pectobacterium*, *Brenneria*, *Ralstonia*, and *Xanthomonas* (Lindgren, 1997). *hrp* genes of *E. amylovora*, *P. syringae*, and *Xanthomonas* spp. are located on the chromosome (Bonas, 1994). However, they are plasmid-borne in *Ralstonia solanacearum* and *P. herbicola* pv. *gypsophila*, and have not been found in non-pathogenic relatives of plant pathogens including several strains of *P. herbicola* and *Pseudomonas fluorescens*. *hrp* genes can be divided into two groups based on Southern hybridization, organization and sequence similarity of secretion genes, presence of common regulatory genes, and complementation assays: group I containing genes of *Erwinia*, *Pantoea*, *Pectobacterium*, *Brenneria* and *P. syringae*, and group II containing those of *R. solanacearum* and *Xanthomonas* (Alfano and Collmer, 1996).

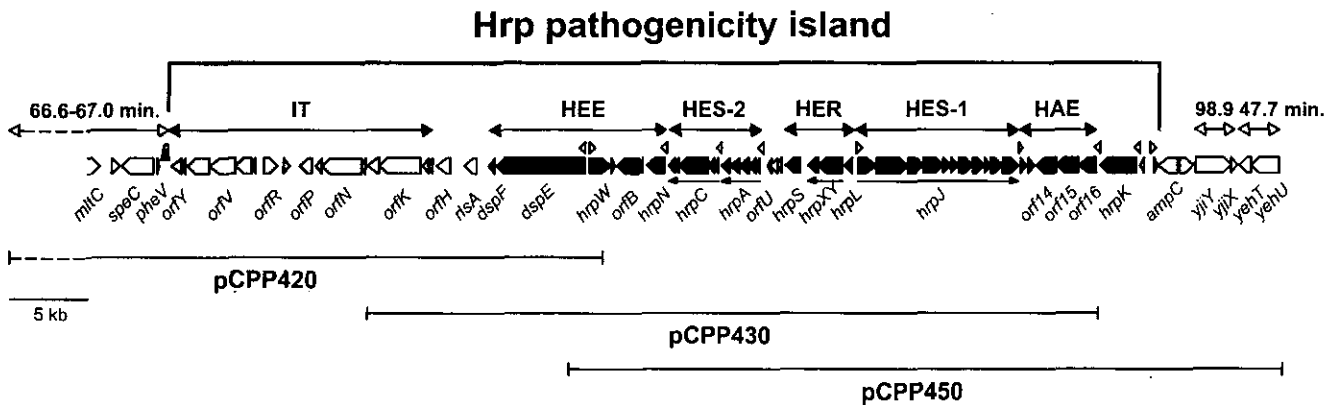
*hrp* genes of *E. amylovora* were first discovered by trans-

poson mutagenesis of strains Ea321 and Ea322 (Bauer and Beer, 1987; Steinberger and Beer, 1988). Subsequently, pCPP430 and pCPP450 carrying Ea321 genomic DNA (Fig. 1) complemented all of the previously obtained *Hrp* mutants of *E. amylovora* and enabled recipient *E. coli* and other non-plant pathogens to elicit the HR in an array of plants, leading to the conclusion that these cosmids harbor the entire *hrp* gene cluster (Beer et al., 1991). These initial experiments have localized the genes required for the *Hrp* phenotype of *E. amylovora* in a ca. 20-25 kb region of DNA. A similar transposon mutagenesis approach was taken for strain CFBP1430 and *hrp* DNA was cloned in several plasmids (Barny et al., 1990; Vanneste et al., 1990). More recently, two cosmids containing a functional *hrp* gene cluster of a *Rubus*-pathogenic strain Ea246 were isolated (Laby, 1997). *hrp* genes of *E. amylovora* can be categorized into three classes based on their roles in the *Hrp* pathogenesis (Kim and Beer, 2000). Regulation genes control the expression of other *hrp* genes; secretion genes encode components of a protein secretion machinery called the *Hrp* (type III) pathway, which is involved in delivering proteins to the cell exterior and inside the host cell; and secreted effector proteins are likely to be responsible for promoting parasitism or eliciting defense responses.

### *Hrp* Pathogenicity Island

In animal pathogens, genes encoding virulence factors are often located in discrete chromosomal segments called “pathogenicity islands (PAIs)”, implying that they have been horizontally transferred from other bacteria (Kaper and Hacker, 1999; Ochman et al., 2000). Like bacteriophages and conjugative transposons, PAIs often utilize highly conserved tRNA genes as a landmark to insert their DNA into the bacterial chromosome. The *hrp*-flanking regions of *E. amylovora* have been recently sequenced, and revealed the presence of *pheV* and a cryptic recombinase gene at one end (Fig. 1; Kim and Beer, manuscript in preparation). Genes likely to be the orthologs of those in *E. coli* were found beyond the tRNA gene; the same was true beyond the other end of the *hrp* gene cluster. Similar but nevertheless not the same findings have been made for the region surrounding the *hrp* gene clusters of *P. syringae* (Alfano et al., 2000) and *Pectobacterium chrysanthemi* (Kim, Collmer and Beer, unpublished results). These observations suggest that the *hrp* gene clusters of these bacteria are parts of large pathogenicity cassettes, which could be called the “*Hrp* PAIs”.

The *Hrp* PAI of *E. amylovora* is more than 60 kilobases in size and harbors as many as 60 open reading frames (ORFs) (Fig. 1; Kim and Beer, manuscript in preparation). It apparently is composed of two large segments: one that is



**Fig. 1.** The Hrp pathogenicity island of *E. amylovora* Ea321. The island consists of loci possibly involved in the island transfer (IT), production of Hrp effectors and elicitors (HEE), Hrp effector secretion (HES), regulation of *hrp* gene expression (HER), and synthesis of putative Hrp-associated enzymes (HAE). Arrow boxes indicate ORFs. ORFs implicated in the Hrp pathogenesis or likely to be part of the *hrp* regulon are shown by filled arrow boxes. Potential HrpL-dependent promoters are indicated by open vertical triangles above the ORFs. The cryptic recombinase gene *orfY* next to *pheV* (lighthouse) marks the “left” end of the island. Outside the island are the regions containing close homologs of *E. coli* (shown by *E. coli* K-12 designations), of which the genomic locations are indicated by map coordinates (min.). pCPP420, pCPP430, and pCPP450 are cosmids clones that were used for sequencing and characterization.

involved in pathogenesis and the other enriched with bacteriophage-related genes. The part for pathogenesis can be further divided into a region containing a regulator of levan production *rlsA*, a region containing the *hrp/dsp* gene cluster, and a region containing genes possibly involved in biosynthesis of a modified tripeptide. Southern hybridization suggested that, while *hrp* genes are present, the recombinase gene found at one end of the *E. amylovora* Hrp PAI is not present in the species of *Pantoea*, *Pectobacterium*, and *Brenneria* (Kim and Beer, manuscript in preparation). This implies that different mobile elements may have been responsible for introduction or movement of Hrp PAIs among plant-pathogenic members of the *Enterobacteriaceae*.

### Regulation of *hrp* Gene Expression

Expression of *E. amylovora hrp* genes is repressed in rich media, while it is induced *in planta* or in minimal media that simulate the conditions of plant apoplasts. The environmental signals that affect gene expression include carbon and nitrogen sources, pH, temperature, and osmolarity (Wei et al., 1992b). HrpL, a member of a subfamily of eubacterial  $\sigma$  factors that regulate extracytoplasmic functions, seems the master switch of the *hrp* systems of *E. amylovora* (Wei and Beer, 1995); it activates all secretory *hrp* operons, harpin genes, and *dsp* genes. HrpL recognizes a conserved sequence motif located at the promoter regions of the HrpL-dependent operons or genes (Xiao and Hutcheson, 1994). Expression of *hrpL* seems to depend on the  $\sigma^{54}$ /HrpS system. A  $\sigma^{54}$  consensus sequence has been found from the promoter region of *hrpL* (Wei et al., 2000), and expression

of *hrpL* is partially controlled by HrpS (Wei and Beer, 1995), which belongs to the NtrC family of  $\sigma^{54}$  enhancer-binding proteins. Two other regulatory proteins, HrpX and HrpY, are required for expression of *hrpL* (Wei et al., 2000). They are members of the two-component regulatory protein family that is widely used for prokaryotic gene expression. HrpX is a putative sensor that likely perceives environmental signals, and HrpY is a potential accompanying response regulator that may transmit the signal from HrpX to *hrpL*. *hrpY* is absolutely required for the Hrp phenotype and *hrpL* expression, whereas *hrpX* is only partially involved in *hrpL* activation.

Other factors might be involved in the regulation of *E. amylovora hrp* genes, too. In *Pectobacterium carotovora* subsp. *carotovora*, *hrpN* is regulated by homoserine lactone and global regulators, RsmA and *rsmB* (Cui et al., 1996; Liu et al., 1998). Also, in *P. syringae* pv. *syringae*, *hrpV* has been shown to negatively regulate *hrp* gene expression (Preston et al., 1998).

### Hrp Type III Protein Secretion

Initial sequencing analysis of *hrp* secretion genes in the early 1990s surprisingly indicated homology with genes of animal-pathogenic bacteria that are involved in secretion of virulence proteins (Van Gijsegem et al., 1993). This novel secretion pathway, distinct from hemolysin secretion and general secretory pathways and evolutionarily related to the flagellar biogenesis system, was designated the “type III” protein secretion system (Hueck, 1998). By the mid-1990s, confusion in *hrp* gene names increased as the number of *hrp* genes identified from different bacteria increased. Thus,

the name *hrc* (for **H**R and **C**onserved; Bogdanove et al., 1996a) was given to nine highly conserved type III secretion genes.

Sequence analysis, comparison with homologs, and experimental evidence suggested that HrcC is an outer-membrane protein, HrcJ is a lipoprotein, HrcR, HrcS, HrcT, HrcU and HrcV are polytopic inner-membrane proteins, and HrcN is a cytoplasmic ATPase homolog. The subcellular location of HrcQ remains unclear, but a homolog in *Salmonella enterica*, SpaO, is exported via the type III pathway. Other less conserved Hrp secretion proteins include HrpQ of *E. amylovora* and *P. syringae* and HrpW of *R. solanacearum*, which are homologous to *Yersinia* YscD (Bogdanove et al., 1996b), and HrpE of *E. amylovora* and *P. syringae*, HrpF of *R. solanacearum*, and HrpB5 of *X. campestris* pv. *vesicatoria*, which are homologous to YscL/FliH (Kim et al., 1997). The HrpQ homolog in the *S. enterica* SPI1 system, PrgH, is a component of the type III secretion structure (Kubori et al., 1998). Among the Hrc proteins, HrcC is worthy of special attention in that homologs are present in the type II secretion system, but not in flagellar export systems. Furthermore, HrcC homologs such as PulD, OutD and pIV form an outer-membrane protein complex called secretin, which functions as a "gate keeper" by specifically recognizing its target proteins. Recent electron microscopy work on the SPI1-encoded type III apparatus of *Salmonella enterica* visualized a supramolecular structure surprisingly similar to the flagellar structure (Kubori et al., 1998).

### Harpins: Hrp-secreted HR Elicitors

Harpins elicit the HR in plants and are first proteins shown to be secreted via the Hrp (type III) pathway (Wei et al., 1992a; Wei and Beer, 1993). They are hydrophilic (acidic), glycine-rich, and lack cysteine. Four classes of harpins (hrpN, HrpW, HrpZ, PopA) have been identified, and no significant amino acid similarities exist between them. Although harpins have been identified based on their ability to elicit the HR when infiltrated into intercellular spaces of plants, their biological function in pathogenesis remains unclear (Alfano and Collmer, 1996). Phenotypes of harpin mutants range from no changes from the wild type to drastic reduction in virulence and HR induction. The "cell-killing" action of harpins does not appear to be due to potential enzymatic or toxic function; rather, the HR-eliciting activity is heat-stable, requires plant metabolism, and fragments of harpins can elicit the response. *E. amylovora* has two harpin genes, *hrpN* and *hrpW*, which are located at one end of the *hrp* gene cluster (Fig. 1; Wei et al., 1992a; Kim and Beer, 1998).

In addition to eliciting the HR, HrpN induces systemic

resistance against a variety of pathogens (Dong et al., 1999), induces repellency against insects (Zitter and Beer, 1998), and promotes the growth of tobacco and several other plants (Qiu et al., 1997). Intriguingly, simple treatment of harpins such as spraying leaf surfaces, drenching roots, or soaking seeds is sufficient for these activities. The mechanisms underlying these phenomena are still unclear, but beneficial effects of harpins are being actively pursued by a company for potential use in agriculture. In addition, an approach to control plant diseases by expressing the *hrpN* gene in plants in a pathogen-inducible fashion is being developed on a number of plant species (Beer et al., unpublished data).

### DspE: a Pathogenicity/Avirulence Effector Protein

In addition to harpins, several other proteins including HrpJ, HrpA, and potential virulence/avirulence effector proteins may be delivered via the Hrp pathway of *E. amylovora*. A number of proteins have been detected in culture supernatants of *E. amylovora* Ea321 and Ea273, but not from their *hrp* secretion mutants (Kim, 1997; Kim and Beer, unpublished results). An interesting Hrp-secreted protein is the pathogenicity protein DspE (Gaudriault et al., 1997; Bogdanove et al., 1998a). *dspE* (disease-specific) is a gene in a two-gene locus required for pathogenicity but not required for elicitation of the HR in nonhosts (Fig. 1). This locus is homologous to the *avrE* locus of *P. syringae* and functions as an avirulence locus when expressed in a pathovar of *P. syringae* that infects soybean (Gaudriault et al., 1997; Bogdanove et al., 1998b). This is the first example in *E. amylovora* that may function in avirulence. The next gene *dspF* is also required for pathogenicity and its protein product seems to play roles in secretion of DspE as a specific chaperone.

Recognition of DspE in soybean suggests the possibility of engineering resistance in hosts of *E. amylovora* by using *dspE* to isolate a corresponding R gene from soybean (or another, more genetically tractable non-host plant) and then transferring the gene to the host species. This would result in a durable resistance of host plants to fire blight since *dspE* is required for causing the disease. Currently, this approach is being undertaken with *Arabidopsis* to isolate an R gene specific to *dspE* (Beer et al., unpublished data).

### A Phytotoxin Produced by *E. amylovora*?

Analysis of ORFs at the HAE locus (Fig. 1; Kim and Beer, manuscript in preparation) suggests that they might be a part of the HrpL regulon since the first and the last ORFs are flanked by two converging HrpL-dependent promoter motifs, and that they encode putative enzymes such as

acetyltransferase, two carboxylase/ligase, and amidinotransferase. These enzymes might be translocated via the Hrp pathway into the plant cytoplasm to change metabolic processes. Another more likely possibility is that they produce a modified tripeptide similar in structure to phaseolotoxin. The amidinotransferase may synthesize, from arginine and lysine, ornithine and homoarginine, which are joined to alanine by the two amino acid ligases. The resulting tripeptide would be then modified by the acetyl transferase. If this possibility turns out to be the case, it will be the first example of a phytotoxin synthesis system physically linked to and controlled by the *hrp* system. It should be reminded that *E. amylovora* or any plant-pathogenic bacterium in the *Enterobacteriaceae* has not been known to produce a phytotoxin.

### Future Directions

Although the accumulated information on the *hrp* system and Hrp-secreted proteins during the past 10 years is daunting, it is just a beginning. We do not know how *hrp* genes are regulated spatially and temporally, how the secretion system works mechanistically, what is the full tally of proteins that are secreted, and what are the precise functions of the secreted proteins. The molecular basis of the host specificity of *E. amylovora* is yet another important question. Answers to these questions will lead us toward an understanding of the basis of fire blight and other bacterial diseases of plants and animals, and ultimately toward applications of more effective controls of the diseases.

### References

- Alfano, J. R., Charkowski, A. O., Deng, W. L., Badel, J. L., Petnicki-Ocwieja, T., van Dijk, K. and Collmer, A. 2000. The *Pseudomonas syringae* Hrp pathogenicity island has a tripartite mosaic structure composed of a cluster of type III secretion genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. *Proc. Natl. Acad. Sci. USA* 97:4856-4861.
- Alfano, J. R. and Collmer, A. 1996. Bacterial pathogens in plants: life up against the wall. *Plant Cell* 8:1683-1698.
- Barny, M. A., Guinebretière, M. H., Marçais, B., Coissac, E., Paulin, J. P. and Laurent, J. 1990. Cloning of a large gene cluster involved in *Erwinia amylovora* CFBP 1430 virulence. *Mol. Microbiol.* 4:777-786.
- Bauer, D. W. and Beer, S. V. 1987. Cloning of *Erwinia amylovora* DNA involved in pathogenicity and induction of the hypersensitive reaction. *Acta Hort.* 217:169-170.
- Beer, S. V., Bauer, D. W., Jiang, X. H., Laby, R. J., Sneath, B. J., Wei, Z.-M., Wilcox, D. A. and Zumoff, C. H. 1991. The *hrp* gene cluster of *Erwinia amylovora*. In: *Advances in Molecular Genetics of Plant-Microbe Interactions*, Vol. 1, ed. by H. Henneke and D. P. S. Verma, pp. 53-60. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Bogdanove, A. J., Bauer, D. W. and Beer, S. V. 1998a. *Erwinia amylovora* secretes DspE, a pathogenicity factor and functional AvrE homolog, through the Hrp (type III secretion) pathway. *J. Bacteriol.* 180:2244-2247.
- Bogdanove, A. J., Beer, S. V., Bonas, U., Boucher, C. A., Collmer, A., Coplin, D. L., Cornelis, G. R., Huang, 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., Kim, J. F. and Beer, S. V. 2000. Disease-specific genes of *Erwinia amylovora*: keys to understanding pathogenicity and potential targets for disease control. In: *Fire Blight and its Causative Agent, Erwinia amylovora*, ed. by J. L. Vanneste, pp. 163-177. CAB International, Wallingford, UK.
- Bogdanove, A. J., Kim, J. F., Wei, Z., Kolchinsky, P., Charkowski, A. O., Conlin, A. K., Collmer, A. and Beer, S. V. 1998b. Homology and functional similarity of an *hrp*-linked pathogenicity locus, *dspEF*, of *Erwinia amylovora* and the avirulence locus *avrE* of *Pseudomonas syringae* pathovar tomato. *Proc. Natl. Acad. Sci. USA* 95:1325-1330.
- 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.
- Cui, Y., Madi, L., Mukherjee, A., Dumenyo, C. K. and Chatterjee, A. K. 1996. The RsmA- mutants of *Erwinia carotovora* subsp. *carotovora* strain Ecc71 overexpress *hrpN<sub>Ech</sub>* and elicit a hypersensitive reaction-like response in tobacco leaves. *Mol. Plant-Microbe Interact.* 9:565-573.
- Dong, H., Delaney, T. P., Bauer, D. W. and Beer, S. V. 1999. Harpin induces disease resistance in *Arabidopsis* through the systemic acquired resistance pathway mediated by salicylic acid and the *NIM1* gene. *Plant J.* 20:207-215.
- Gaudriault, S., Malandrin, L., Paulin, J.-P. and Barny, M.-A. 1997. DspA, an essential pathogenicity factor of *Erwinia amylovora* showing homology with AvrE of *Pseudomonas syringae*, is secreted via the Hrp secretion pathway in a DspB-dependent way. *Mol. Microbiol.* 26:1057-1069.
- Geider, K. 2000. Exopolysaccharides of *Erwinia amylovora*: structure, biosynthesis, regulation, role in pathogenicity of amylovoran and levan. In: *Fire Blight: the Disease and its Causative Agent, Erwinia amylovora*, ed. by J. L. Vanneste, pp. 117-140. CAB International, Wallingford, UK.
- Goodman, R. N. and Novacky, A. J. 1994. The hypersensitive reaction in plants to pathogens: a resistance phenomenon. APS Press, 256pp.
- Hueck, C. J. 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* 62:379-433.
- Kaper, J. B. and Hacker, J. 1999. *Pathogenicity Islands and Other Mobile Virulence Elements*. ASM Press, Washington, D. C.,

- USA, 375 pp.
- Kim, J. F. 1997. Molecular Characterization of a Novel Harpin and Two *hrp* Secretory Operons of *Erwinia amylovora*, and a *hrp* Operon of *E. chrysanthemi*. Ph. D. Thesis, Cornell University, Ithaca, New York, USA.
- Kim, J. F. and Beer, S. V. 1998. HrpW of *Erwinia amylovora*, a new harpin that contains a domain homologous to pectate lyases of a distinct class. *J. Bacteriol.* 180:5203-5210.
- Kim, J. F. and Beer, S. V. 2000. *hrp* genes and harpins of *Erwinia amylovora*: a decade of discovery. In: *Fire Blight and its Causative Agent, Erwinia amylovora*, ed. by J. L. Vanneste, pp. 141-161. CAB International, Wallingford, UK.
- Kim, J. F., Wei, Z.-M. and Beer, S. V. 1997. The *hrpA* and *hrpC* operons of *Erwinia amylovora* encode components of a type III pathway that secretes harpin. *J. Bacteriol.* 179:1690-1697.
- Kubori, T., Matsushima, Y., Nakamura, D., Uralil, J., Lara, T. M., Sukhan, A., Galan, J. E. and Aizawa, S. I. 1998. Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* 280:602-605.
- Laby, R. J. 1997. Molecular Studies on Interactions between *Erwinia amylovora* and Its Host and Non-host Plants. Ph.D. Thesis, Cornell University, Ithaca, New York, USA.
- Lindgren, P. B. 1997. The role of *hrp* genes during plant-bacterial interactions. *Annu. Rev. Phytopathol.* 35:129-152.
- Liu, Y., Cui, Y., Mukherjee, A. and Chatterjee, A. K. 1998. Characterization of a novel RNA regulator of *Erwinia carotovora* ssp. *carotovora* that controls production of extracellular enzymes and secondary metabolites. *Mol. Microbiol.* 29:219-234.
- Ochman, H., Lawrence, J. G. and Groisman, E. A. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405:299-304.
- Preston, G., Deng, W. L., Huang, H. C. and Collmer, A. 1998. Negative regulation of *hrp* genes in *Pseudomonas syringae* by HrpV. *J. Bacteriol.* 180:4532-4537.
- Qiu, D., Wei, Z.-W., Bauer, D. W. and Beer, S. V. 1997. Treatment of tomato seed with harpin enhances germination and growth and induces resistance to *Ralstonia solanacearum*. *Phytopathology* 87:S80.
- Steinberger, E. M. and Beer, S. V. 1988. Creation and complementation of pathogenicity mutants of *Erwinia amylovora*. *Mol. Plant-Microbe Interact.* 1:135-144.
- van der Zwet, T. and Beer, S. V. 1999. Fire Blight and Its Nature, Prevention and Control: a Practical Guide to Integrated Disease Management. U.S. Department of Agriculture, Washington, D. C., USA, 83 pp.
- 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.
- Vanneste, J. L. 2000. Fire Blight and its Causative Agent, *Erwinia amylovora*. CAB International, Wallingford, UK, 370 pp.
- Vanneste, J. L., Paulin, J.-P. and Expert, D. 1990. Bacteriophage Mu as a genetic tool to study *Erwinia amylovora* pathogenicity and hypersensitive reaction on tobacco. *J. Bacteriol.* 172:932-941.
- Wei, Z.-M. and Beer, S. V. 1993. HrpI of *Erwinia amylovora* functions in secretion of harpin and is a member of a new protein family. *J. Bacteriol.* 175:7958-7967.
- Wei, Z.-M. 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.
- Wei, Z., Kim, J. F. and Beer, S. V. 2000. Regulation of *hrp* genes and type III protein secretion in *Erwinia amylovora* by HrpX/HrpY, a novel two-component system, and HrpS. *Mol. Plant-Microbe Interact.* 13:1251-1262.
- Wei, Z.-M., Laby, R. J., Zumoff, C. H., Bauer, D. W., He, S. Y., Collmer, A. and Beer, S. V. 1992a. Harpin, elicitor of the hypersensitive response produced by the plant pathogen *Erwinia amylovora*. *Science* 257:85-88.
- Wei, Z.-M., Sneath, B. J. and Beer, S. V. 1992b. Expression of *Erwinia amylovora* *hrp* genes in response to environmental stimuli. *J. Bacteriol.* 174:1875-1882.
- Xiao, Y. and Hutcheson, S. W. 1994. A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae*. *J. Bacteriol.* 176:3089-3091.
- Zitter, T. A. and Beer, S. V. 1998. Harpin for insect control. *Phytopathology* 88:S104-S105.