The Plant Cell, Vol. 8, 1683–1698, October 1996

Bacterial Pathogens in Plants: Life up against the Wall

James R. Alfano and Alan Collmer[1]

Department of Plant Pathology, Cornell University, Ithaca, New York 14853-4203

INTRODUCTION

Higher plants contain potentially vast sources of nutrients for the myriad bacterial species in their environment, and most bacteria are small enough to pass through stomates and other natural openings into the apoplast—the anteroom for these riches. However, surprisingly few bacteria raid the nutrient stores of living plant cells, apparently because the metabolic intimacy involved in parasitism requires the work of specialists. Of these specialists, some in the Rhizobiaceae produce hypertrophies that are genetically engineered or developmentally tricked into providing an undefended, nutritive niche in root cortical tissues and rhizospheres (see Long, 1996; Sheng and Citovsky, 1996, in this issue), whereas others, mostly Gram-negative bacteria in the Pseudomonadaceae and Enterobacteriaceae, specialize in colonizing the apoplast.

It is the apoplastic colonizers that are the common pathogens that produce the rots, spots, wilts, cankers, and blights afflicting virtually all crop plants, and their relationship with the host is defined by two features. They spend their parasitic life up against the wall of plant cells, in the intercellular spaces of various plant organs or in the xylem, and they are necrogenic—able to cause the death of plant cells. Their ability to multiply and then sooner or later to kill plant cells depends on secreted enzymes that degrade the wall or on molecules that pass through it. This review addresses our progress in understanding this molecular traffic and how it may enable necrogenic bacterial pathogens to colonize the apoplast.

The present picture of pathogenesis has been strongly determined by three developments. The first was the discovery that bacteria elicited the defense-associated hypersensitive response (HR) in plants during incompatible interactions. The HR was first observed as a rapid localized collapse of tobacco leaf tissue after infiltration of high numbers of bacterial pathogens that are host specific for other plant species (Klement, 1963; Klement et al., 1964). Because the ability to elicit the HR is a unique attribute of the necrogenic pathogens and these bacteria can avoid or suppress its elicitation in their hosts, the HR phenomenon appears central to bacterial pathogenicity and host specificity and has attracted much attention (Klement, 1982; Goodman and Novacky, 1994; see also Dangl et al., 1996; Hammond-Kosack and Jones, 1996, in this issue). The second development was the application of the molecular tools of transposon mutagenesis, broad-host-range cosmid vectors, and marker-exchange mutagenesis to identify and manipulate bacterial genes that have a readily scored phenotype when mutated, conjugated into a related strain, or expressed in *Escherichia coli*. These approaches have yielded a large inventory of *hrp* (hypersensitive response and pathogenicity) and *avr* (avirulence) genes that directly relate to the HR puzzle as well as numerous other genes associated with pectic enzyme, toxin, and extracellular polysaccharide (EPS) production. Rather than detail this inventory (which may be fundamentally incomplete; see below), we use representative components to develop a model for bacterial plant pathogenesis that is based on the very recent third development—the discovery that the *hrp* genes encode a protein secretion system, shared in plant and animal pathogens, that has the potential to transfer virulence proteins into eukaryotic host cells.

The necrogenic bacteria have diverse pathogenic personalities with a bewildering array of symptoms and host specificities. The growing evidence that the *hrp* genes are ubiquitous in these pathogens, controlling early (and generally essential) interactions with plants, provides a unifying entry point for exploring bacterial phytopathogenicity. Hence, after introducing the representative pathogens, we explore the dynamic operation of the Hrp system and then turn briefly to factors such as toxins, EPS, and pectic enzymes that affect the full development of plant disease.

MODEL PATHOGENS AND INTERACTIONS

Key characteristics of several model Gram-negative phytopathogens are shown in Table 1. These bacteria are all capable of causing necrosis, but their necrogenic aggressiveness varies. Brute-force, necrotrophic pathogens rapidly kill parenchymatous tissues during active pathogenesis, whereas stealthy, biotrophic pathogens characteristically multiply in host tissues for some period before causing any necrosis (Collmer and Bauer, 1994). The HR is elicited by the biotrophic pathogens during incompatible interactions with nonhosts, but *Erwinia chrysanthemi* mutants with a reduced pectolytic capacity can also elicit a typical HR that is independent of host range (Bauer et al., 1994). Strains in *Xanthomonas campestris* and *Pseudomonas syringae* are assigned to pathovars

[1] To whom correspondence should be addressed.

Table 1. Model Necrogenic Gram-Negative Phytopathogens

Pathogen	Host Range; Model Hosts	Typical Diseases	Phenotype of *hrp* (Type III Secretion) Mutants[a]	Phenotype of Type II Secretion Mutants[b]	Other Disease Factors
Necrotrophic					
Erwinia carotovora and *E. chrysanthemi*[c]	Wide; potato, tobacco seedlings, Saintpaulia	Soft rots	HR^{-d}; infectivity reduced but wild-type maceration	No maceration	Pectic enzymes; siderophores; autoinduction
Biotrophic					
E. amylovora	Rosaceae; apple and pear	Fire blight	Hrp^{-d}	Not known	EPS; harpin
E. stewartii	Maize	Stewart's wilt	Wts^{-d}	Not known	EPS; autoinduction
Ralstonia solanacearum[e]	Solanaceae; tomato and tobacco	Wilts	Hrp^-	Virulence reduced	EPS; volatile signal and global regulation
Xanthomonas campestris pathovars	Individually narrow; pepper, tomato, brassicas	Foliar spots and blights	Hrp^-	Virulence reduced	Avr proteins; global regulation
Pseudomonas syringae pathovars	Individually narrow; tomato, Arabidopsis, legumes	Foliar spots and blights	Hrp^-	Not known	Avr proteins; toxins

[a] Harpins are the only proteins directly shown to travel via this pathway; evidence for Avr protein traffic is discussed in the text. The virulence phenotype reflects the collective contribution of all proteins traveling the pathway. For references, see Bauer et al. (1994) regarding *E. chrysanthemi* type III mutants and those in Bonas (1994) for all other bacteria.

[b] Virtually all plant cell wall–degrading enzymes travel this pathway. For references, see Kang et al. (1994) and those in Salmond (1994).

[c] *E. chrysanthemi* has been the model for the Hrp system; *E. carotovora* for autoinduction.

[d] HR^- denotes loss of HR elicitation activity in these bacteria; Hrp^- denotes loss of HR and parasitism/pathogenicity; Wts^- denotes lack of water-soaked lesions.

[e] Synonyms are *Pseudomonas solanacearum* and *Burkholderia solanacearum* (Yabuuchi et al., 1992, 1995).

based on host specificity and associated phenotypic characteristics and sometimes to races within pathovars based on interactions with differential cultivars of the host. For example, *X. campestris* pv *campestris* causes black rot of crucifers, and *P. syringae* pv *glycinea* causes bacterial blight of soybean, but both elicit the HR in tobacco. Table 1 also highlights the importance of two protein secretion pathways in the virulence of these bacteria and indicates other specific factors that are discussed below.

Much research has focused on the differing interactions between plants and biotrophic pathogens (compatible and incompatible) and nonpathogens. These interactions are summarized in Figure 1. The HR is the most dramatic of these responses, and several additional observations are important in considering its nature. First, the macroscopically observable HR requires high levels of bacteria (>5 × 10^6 cells/mL) because it results from single bacteria eliciting death in single plant cells in a one-to-one manner, and a threshold level of individual cell deaths is required for tissue death (Turner and Novacky, 1974). Second, HR elicitation appears to require contact between plant and bacterial cells that are both metabolically active and synthesizing new proteins (Holliday et al., 1981; Klement, 1982). Although tissue collapse and death may not occur until 12 to 36 hr postinoculation, antibiotic treatment experiments suggest that bacteria may deliver the HR elicitation signal within a few minutes of contact (Huynh et al., 1989). Third, the HR appears to represent programmed cell death (He et al., 1993; Dietrich et al., 1994; Greenberg et al., 1994), but the signal transduction events and mechanisms underlying this process are still unknown (see Dangl et al., 1996, in this issue).

Although several plant responses are consistently associated with incompatible interactions and the HR (Figure 1), their actual roles are not clear. For example, the data are either lacking

or conflicting regarding (1) the causal relationship between active oxygen generation and HR elicitation (Levine et al., 1994; Glazener et al., 1996; see also Hammond-Kosack and Jones, 1996, in this issue), (2) the relationship between the HR and the XR (K^+ efflux/H^+ influx exchange response; Atkinson, 1993; He et al., 1994), and (3) the role in defense of antimicrobial phytoalexins (Long et al., 1985; Pierce and Essenberg, 1987; Glazebrook and Ausubel, 1994) and pathogenesis-related proteins (see Ryals et al., 1996, in this issue). However, the XR may be particularly important in compatible (disease-causing) interactions because alkalinization of the apoplast has been shown to foster both sucrose leakage from plant cells and bacterial multiplication (Atkinson and Baker, 1987a, 1987b).

As suggested by the different response patterns outlined in Figure 1, the fate of plant–bacterium interactions may be determined very early after inoculation. When considering possible determinative factors, it is useful to keep in mind that compatible pathogens, which appear to be able to suppress rapid, "weak" defense responses, can promote the growth of nonpathogens, whereas coinoculation of compatible and incompatible pathogens results in incompatibility unless the compatible pathogen has been given a substantial head start (Young, 1974; Klement, 1982; Jakobek et al., 1993). Of course, a critical decision in the interaction is whether or not the HR is triggered, and much of the remainder of this article concerns the bacterial factors involved in HR elicitation.

THE HRP SYSTEM UNDERLYING BASIC PATHOGENICITY

hrp Genes

The ability of the necrogenic phytopathogens to elicit the HR resides in *hrp* genes, which were first found in *P. syringae* pv *syringae* and *P. syringae* pv *phaseolicola* by identifying Tn*5* transposon mutants that grew normally in minimal media but failed to elicit the HR in nonhost tobacco or cause disease or multiply in host bean (Niepold et al., 1985; Lindgren et al., 1986). Thus, *hrp* mutants behave essentially like nonpathogens in plants. *hrp* genes are clustered and are likely to occur within "pathogenicity islands" containing supporting virulence genes (e.g., Lorang and Keen, 1995). The *hrp* clusters of *P. s. syringae* 61 and *E. amylovora* Ea321, carried on recombinant cosmids pHIR11 and pCPP430, respectively, enable nonpathogenic bacteria such as *P. fluorescens* and *E. coli* to elicit the HR (but not disease) in tobacco and many other plants (Huang et al., 1988; Beer et al., 1991).

Initial DNA sequencing of the *hrp* clusters of *Ralstonia solanacearum* GMI1000, *X. c. vesicatoria* 85-10, and *P. s. syringae* 61 revealed homologies with components of the virulence protein (Yop) secretion system in animal pathogenic *Yersinia* spp (Fenselau et al., 1992; Gough et al., 1992; Huang et al.,

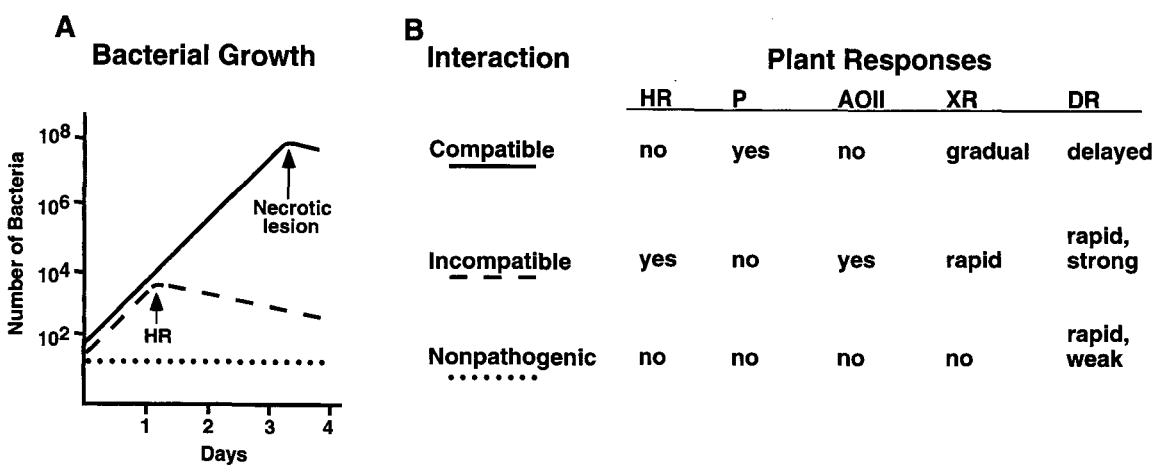

Figure 1. Typical Interactions between Compatible and Incompatible Biotrophic Pathogens, or Nonpathogens, and Plants.

(A) Generalized bacterial population dynamics graphically relate the potential to elicit necrosis and the ability to multiply in plants, and they show that multiplication ceases upon actual development of the necrosis associated with either the HR or disease lesions (Klement, 1982).

(B) Interaction classes are defined by the differing bacterial growth patterns and by the suites of plant responses. HR is further described in the text. P denotes the development of lesions and other symptoms that accompany pathogenesis. AOII denotes a sustained generation of active oxygen that occurs 1.5 to 3 hr after inoculation (AOI is a brief nonspecific response immediately after inoculation; reviewed in Baker and Orlandi, 1995; see also Dangl et al., 1996; Hammond-Kosack and Jones, 1996, in this issue). XR denotes a K^+ efflux/H^+ influx that occurs simultaneously with AOII in incompatible interactions (reviewed in Atkinson, 1993). DR denotes the expression of a variety of defense-response genes, particularly those directing the synthesis of phenylpropanoid pathway enzymes and their phytoalexin products, which occurs rapidly (within 6 hr) except in compatible interactions, where it can be delayed for several days (Jakobek and Lindgren, 1993; Meier and Slusarenko, 1993).

1992), thereby establishing the existence of the conserved "type III" secretion system in Gram-negative bacteria (Salmond and Reeves, 1993; Van Gijsegem et al., 1993). The near completion of the *hrp* cluster sequences in these three phytopathogens and in *E. amylovora* Ea321 has revealed that the homologies with type III protein secretion system components in animal pathogenic *Yersinia*, *Shigella*, and *Salmonella* spp are extensive (Huang et al., 1993; Lidell and Hutcheson, 1994; Fenselau and Bonas, 1995; H.-C. Huang et al., 1995; Preston et al., 1995; Van Gijsegem et al., 1995; Bogdanove et al., 1996b). This has led to nomenclatural changes and refinement of the *hrp* gene concept (Bogdanove et al., 1996a). The nine *hrp* genes that are broadly conserved in plant and animal pathogens have been redesignated as *hrc* (hypersensitive response and conserved) and given the last letter assignment of their *Yersinia ysc* (Yop secretion) homologs. The *hrp* genes, and particularly the *hrc* subset, are now considered to be fundamentally involved in type III protein secretion in phytopathogenic bacteria.

The type III secretion system appears to have been acquired by horizontal transfer in a variety of pathogenic bacteria (Groisman and Ochman, 1993; Barinaga, 1996). Within the phytopathogens, comparisons of *hrp* gene sequences (Bogdanove et al., 1996b), *hrp* gene arrangements (Fenselau and Bonas, 1995; H.-C. Huang et al., 1995; Van Gijsegem et al., 1995; Bogdanove et al., 1996b), and *hrp* regulatory elements (discussed below) reveal two groups. Group I contains *P. syringae* and *E. amylovora*; group II contains *R. solanacearum* and *X. c. vesicatoria*. The discrepancy between the *hrp* gene similarity groups and taxonomic relationships is consistent with horizontal acquisition of the system by phytopathogens.

The Hrp (Type III) Protein Secretion System and Its Regulation

The type III secretion pathway is one of at least three distinct pathways that Gram-negative bacteria use to secrete proteins across their inner and outer membranes (Salmond and Reeves, 1993). It is unique among these secretion pathways in its ability to deliver virulence proteins directly into host cells (Rosqvist et al., 1994; Sory and Cornelis, 1994). In *Yersinia*, *Shigella*, and *Salmonella* spp, it appears that the pathway can direct proteins into either the extracellular milieu or host cells. Proteins that are secreted into the milieu may regulate the secretion pathway or form extracellular components of the secretion apparatus (and may also have a direct role in virulence). Proteins that are transferred into host cells appear to be important virulence factors (reviewed in Galan, 1996). In plant pathogens, harpin proteins are known to be secreted into the milieu by the Hrp pathway, and there is evidence that Avr proteins are transferred into plant cells.

Eight of the nine Hrc proteins are homologous to proteins involved in the biogenesis of bacterial flagella and the secretion of flagellar-specific proteins. This is likely important because the flagellar system supports highly regulated protein secretion events involving ordered translocation of different proteins, release of measured protein "doses," and formation of extracellular appendages, all of which may serve the proper delivery of virulence proteins into host cells (Macnab, 1996).

Unfortunately for researchers, proteins targeted to the host via the type III pathway may elude identification for two reasons. First, secretion via this flagellar-derived system is independent of the general export (Sec) system; hence, these proteins lack N-terminal signal peptides (or any other shared feature yet identified from their sequences) that would reveal them as targeted for secretion. Second, the secretion of many of these proteins does not appear to occur in culture because it is dependent on contact with host cells (Rosqvist et al., 1994; Galan, 1996).

Regulation of *hrp* gene expression offers further clues to Hrp function in these bacteria. With the possible exception of the necrotroph *E. chrysanthemi* (Collmer et al., 1994), *hrp* genes are not expressed in rich media (Bonas, 1994). Rather, they are most strongly expressed in various minimal media that mimic plant apoplastic fluids, particularly media deficient in organic nitrogen (Huynh et al., 1989; Arlat et al., 1992; Rahme et al., 1992; Schulte and Bonas, 1992; Wei et al., 1992b; Xiao et al., 1992). No plant inducers of the *hrp* genes have been identified, and *hrp*-dependent elicitation of the HR in nonhosts argues against host-specific *hrp* gene induction.

The genetics of *hrp* regulation are surprisingly different in bacteria harboring the group I and II Hrp systems. In group I, *hrp* expression is dependent on HrpL, a member of the ECF (extra cytoplasmic function) family of sigma factors (Xiao and Hutcheson, 1994; Xiao et al., 1994; Wei and Beer, 1995). *hrpL* expression, although normally dependent on HrpR and HrpS, can be manipulated experimentally to permit useful hyperexpression of the *hrp* regulon (Grimm and Panopoulos, 1989; Xiao et al., 1994; Grimm et al., 1995). In the group II system, *R. solanacearum hrp* expression is dependent on HrpB, a member of the AraC family of positive activators, and the homologous HrpX appears to have the same function in *Xanthomonas* spp (Genin et al., 1992; Oku et al., 1995; Wengelnkik and Bonas, 1996). All of these regulatory proteins have been found through the Hrp^- phenotype of respective mutants, and additional regulatory genes with more subtle phenotypes likely await discovery.

PROTEINS DELIVERED BY THE HRP SYSTEM

Harpins

Harpins are glycine-rich, cysteine-lacking proteins that are secreted in culture when the Hrp system is expressed and that possess heat-stable HR elicitor activity when infiltrated at relatively high concentrations (> 0.1 μM) into the leaves of tobacco and several other plants. This broad definition can encompass

the products of the *E. amylovora* $hrpN_{Ea}$ (Wei et al., 1992a), *E. chrysanthemi* $hrpN_{Ech}$ (Bauer et al., 1995), *P. syringae hrpZ* (He et al., 1993), and *R. solanacearum popA* (Arlat et al., 1994) genes, the first bacterial proteins shown to have HR elicitor activity. Despite these unifying properties, the harpin proteins of *Erwinia* and *Pseudomonas* do not appear to be homologous, mutations in their respective genes have very different pathogenic effects, harpins have yet to be found in *Xanthomonas* spp, and the primary function of the known harpins is unclear. They could, for example, serve parasitism directly by eliciting alkalinization of the apoplast and nutrient release. Alternatively, they could act indirectly by assisting the delivery of other bacterial proteins to plant cells. The ability of the purified harpins of *E. amylovora* and *P. syringae* to elicit both alkalinization of suspension-cultured plant cells and apparent programmed cell death in leaf tissues supports a direct action (Wei et al., 1992a; He et al., 1993, 1994), but mutant phenotypes described below challenge this concept.

Harpin activity does not appear to be enzymatically based because elicitor activity survives heat treatment and the deletion of major portions of the protein (Wei et al., 1992a; He et al., 1993; Arlat et al., 1994; Alfano et al., 1996). There is also no evidence that the harpins of *E. amylovora*, *E. chrysanthemi*, and *P. syringae* directly control host range (Wei et al., 1992a; Bauer et al., 1995). For example, the HrpZ proteins from *P. syringae* pvs *syringae*, *glycinea*, and *tomato* are all active on tobacco (nonhost) and tomato (host for *P. s. tomato*) but not soybean (host for *P. s. glycinea*; Preston et al., 1995). In contrast, PopA3 isolated from *R. solanacearum* has elicitor activity in nonhost tobacco and resistant petunia genotypes but not in host tomato or susceptible petunia genotypes (Arlat et al., 1994).

Postulating a general model for the role of harpins in elicitation of the HR is further complicated by the range of phenotypes exhibited by harpin mutants. For example, harpin gene mutations have strong HR-reduced phenotypes in *E. amylovora* Ea321 (Wei et al., 1992a) and *E. chrysanthemi* (Bauer et al., 1995) but weak or no phenotype in *E. amylovora* CFBP1430 (Barny, 1995), *P. syringae* (Alfano et al., 1996), and *R. solanacearum* (Arlat et al., 1994). The HR phenotypes of *hrp*-related mutations in pHIR11, which carries the functional *P. s. syringae* 61 *hrp* cluster, further confound the harpin elicitor concept. *E. coli* cells carrying pHIR11 with Δ*hrpZ* mutations elicit only a weak and spotty HR (Alfano et al., 1996), but similar mutants hyperexpressing the *hrp* genes because of constitutive expression of *hrpL* in *trans* elicit a strong HR (Pirhonen et al., 1996). More puzzling, *P. fluorescens* carrying pHIR11 with *hrmA* mutations secretes wild-type levels of HrpZ without eliciting any necrosis (Alfano et al., 1996). Thus, purified HrpZ is sufficient to elicit an apparent programmed cell death that is indistinguishable from the HR elicited by bacteria (He et al., 1993), but HrpZ is insufficient for the bacteria themselves to elicit the HR (Alfano et al., 1996).

Interestingly, the *hrmA* gene is not required for *P. s. syringae* 61 itself to elicit the HR in tobacco; it is present in only a few strains of *P. syringae*, and furthermore, it is located at the edge of the *hrp* cluster in the same position as an *avr* gene in *P. s. phaseolicola* 1302A (Huang et al., 1991; Heu and Hutcheson, 1993; Mansfield et al., 1994). This suggests that at least in *P. syringae*, Avr proteins (which HrmA appears to be) may be more important than harpins in eliciting the HR.

Avr Proteins

avr genes control host specificity in *P. syringae* and *X. campestris* at the race–cultivar level by triggering the HR when the host carries a corresponding resistance (*R*) gene, in accordance with Flor's gene-for-gene (*avr*-for-*R*) hypothesis (Flor, 1956; Keen, 1990; see also Crute and Pink, 1996; Dangl et al., 1996; Hammond-Kosack and Jones, 1996, in this issue). Thus, in fundamental contrast to the *hrp* genes, *avr* genes are characteristically scattered in their distribution among strains of phytopathogenic bacteria (Dangl, 1994; Leach and White, 1996).

avr genes are typically identified by screening a broad-host-range cosmid library of donor race DNA in a recipient race that is normally virulent (compatible) on tester plant cultivars that are incompatible with the donor. Conversion of the pathogen reaction from virulence to avirulence on the tester cultivars defines the presence and identity of the cloned *avr* gene in the bacterium (Staskawicz et al., 1984; Keen, 1990). *avr* gene screens involving donor and recipient strains in different pathovars reveal that *P. syringae* and *X. campestris* strains also carry *avr* genes with the potential to interact with *R* genes in nonhosts and thus the potential to control host range at the pathovar–host species level in addition to the more narrow race–cultivar level (Whalen et al., 1988; Kobayshi et al., 1989).

Over 30 *avr* genes have been cloned so far from *P. syringae* and *X. campestris* pathovars by this process, and it is likely that there are many more. Because *avr* genes and their products are treated comprehensively in recent reviews (Dangl, 1994; Leach and White, 1996), our discussion here is limited to an essential overview and current insights leading to a new model for their action.

Unlike harpins, the Avr proteins (and HrmA) reveal no defining physical characteristics. Furthermore, they have no effect when infiltrated into plants, no known biochemical activity (except *P. s. tomato* AvrD), and their sequences do not suggest any function (except *X. c. vesicatoria* AvrBs2). AvrD directs the synthesis of syringolide elicitors of a genotype-specific HR (Keen et al., 1990; Midland et al., 1993). The deduced sequence of the AvrBs2 protein reveals similarity with both *Agrobacterium tumefaciens* agrocinopine synthase (which directs transformed plant cells to produce a carbon source that *A. tumefaciens* utilizes; see Sheng and Citovsky, 1996, in this issue) and *E. coli* glycerophosphoryl diester phosphodiesterase, enzymes catalyzing the synthesis or hydrolysis of phosphodiester linkages (Swords et al., 1996).

Whatever the actions of Avr proteins, they are Hrp dependent. One factor contributing to the *hrp* dependency of *avr*

genes in *P. syringae* is their inclusion in the *hrp* regulon (Huynh et al., 1989; Innes et al., 1993; Shen and Keen, 1993; Xiao and Hutcheson, 1994). However, even when expressed from vector promoters, *avr* genes fail to elicit a genotype-specific HR in *hrp* mutants (Dangl, 1994). An underlying Avr function in Hrp-mediated pathogenesis is suggested by this regulation and by the demonstrable requirement for full virulence in compatible hosts of several *avr* genes (Dangl, 1994; Lorang et al., 1994; Ritter and Dangl, 1995; Yang et al., 1996).

Avr Protein Action within Plant Cells

One of the most puzzling aspects of Avr proteins has been their site of action. They have never been observed to be secreted from the bacterial cytoplasm, even in infected plants (Brown et al., 1993; Young et al., 1994), but there are many arguments (discussed below) against their action in that location. Recent data suggest that at least some Avr proteins act inside plant cells after delivery by the Hrp secretion system. First, the functional cluster of *P. s. syringae* 61 *hrp* genes carried on pHIR11 is sufficient to enable nonpathogens such as *E. coli* and *P. fluorescens* to elicit a genotype-specific HR that is dependent on several *P. s. glycinea* and *P. s. tomato avr* genes (Gopalan et al., 1996; Pirhonen et al., 1996). Moreover, the delivery of these Avr signals is absolutely dependent on Hrp secretion functions and variably dependent on HrpZ. Second, the requirements for the Hrp secretion system and HrpZ in elicitation of a genotype-specific HR cannot be alleviated by *avrB* hyperexpression or exogenous HrpZ or AvrB, but they can be alleviated by expression of *avrB* within plant cells (Gopalan et al., 1996).

The action of AvrB in plant cells was demonstrated with Arabidopsis ecotype Columbia plants carrying the *RPM1 R* gene, which interacts with *avrB* to trigger genotype-specific incompatibility (Bisgrove et al., 1994). An Arabidopsis *rpm1* mutant was transformed with constructs expressing *avrB* and crossed with the wild type. F_1 seedlings carrying both *avrB* and *RPM1* exhibited extensive necrosis on cotyledon leaves 10 days postgermination (Gopalan et al., 1996). Interestingly, the only symptomless transformants obtained in the *rpm1* mutant were those in which AvrB was produced at a low level and with a signal peptide sequence such that the protein would be present in the plant cytoplasm only transiently, suggesting that plants may be exquisitely sensitive to Avr proteins. To express *avrB* without a signal peptide in Arabidopsis, a biolistic cobombardment assay similar to that devised by Mindrinos et al. (1994) was used. Wild-type and *rpm1* mutant leaves biolistically cobombarded with plasmids expressing a β-glucuronidase (*GUS*) reporter and *avrB* failed to produce GUS activity only when *RPM1* and *avrB* were present in the leaf. Thus, both stable and transient expression of *avrB* in Arabidopsis resulted in *RPM1*-dependent necrosis.

Many characteristics of the HR elicitation process can be explained by Hrp-mediated delivery of Avr proteins into plant cells, including: (1) the one-to-one relationship between bacterial cells and HR-responding plant cells (not expected with a diffusible bacterial factor that could affect many plant cells); (2) the determination of genotype-specific avirulence by single bacterial genes in "gene-for-gene" interactions (not expected if multistep biosynthetic pathways producing low molecular weight elicitors were involved); (3) the observation that the *P. syringae* pv *maculicola avrRpt2* and *avrRpm1* gene products interfere with each other extracellularly, as indicated by the development of a genotype-characteristic HR after mixed bacterial inoculations (Reuber and Ausubel, 1996; Ritter and Dangl, 1996); (4) the presence of putative plant nuclear localization signals in members of the AvrBs3 family in *Xanthomonas* spp (Yang and Gabriel, 1995); and (5) the predicted cytoplasmic localization of the products of the *R* genes *PTO*, *RPS2*, and *RPM1*, which interact with *P. syringae avr* genes (Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995; Staskawicz et al., 1995; see also Bent, 1996, in this issue).

There are several potential reasons why it has not been possible to detect the transfer of Avr proteins from bacteria to plant cells. These include the following: the likely host contact-dependent operation of the type III secretion system (predicted from observations with the animal pathogens); the fact that the volume of a plant cell is four orders of magnitude greater than that of a bacterial cell; and the possibility that transfer of Avr proteins may be transient, involve a small fraction of the bacterial pool, and be followed by rapid degradation in the host. Similarly, evidence for the transfer of VirD2 and VirE2 from *A. tumefaciens* to plant cells is indirect at this point, although transfer of the T-DNA complex demonstrates that the plant cell wall is not an impenetrable barrier to specialized bacterial protein transfer systems (Zupan and Zambryski, 1995; see also Sheng and Citovsky, 1996, in this issue).

A Model for Plant-Bacterium Interactions and Coevolution Based on Hrp Delivery of Avr Proteins into Plant Cells

Figure 2 presents a proposed model for the interaction of a necrogenic bacterial pathogen with a plant cell, in which the delivery of Avr proteins is the central parasitic event. According to this model, there may be many Hrp-delivered proteins with a primary function in parasitism, only a subset of which will have Avr phenotypes in some plants. A priori, we can expect these proteins to have two general functions: to defeat host defenses and to locally modify the apoplast for bacterial colonization through nutrient release, watersoaking, and pH increase (Collmer and Bauer, 1994). We may further assume that many of these proteins would most efficiently exploit the proposed direct access to the host metabolic machinery by manipulating signal transduction and gene regulation events (e.g., AvrBs3 with its putative nuclear localization signals). Others may divert host metabolic energy to the production and release of nutrients for bacterial consumption in the apoplast

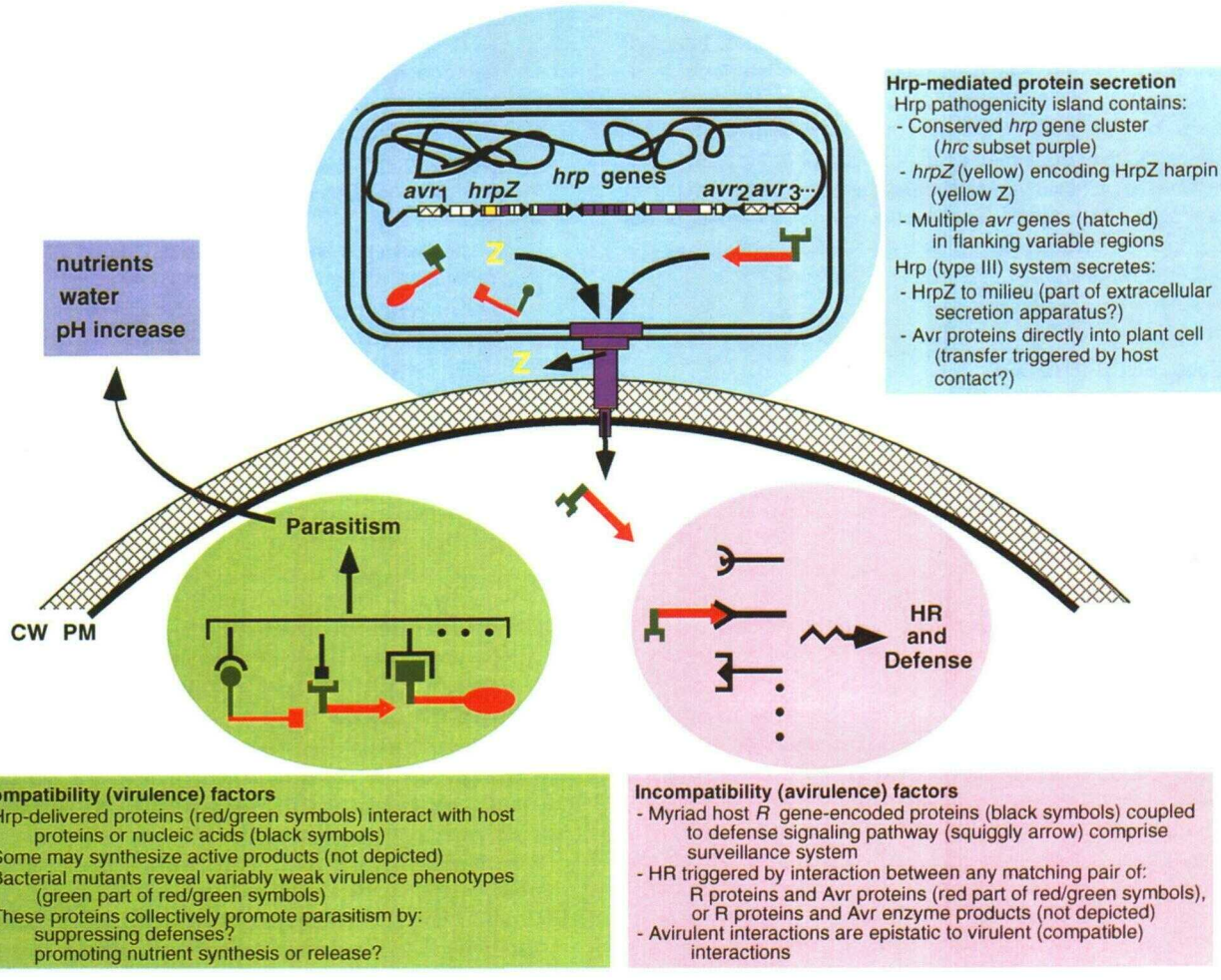

Figure 2. Proposed Model of Bacterial Pathogenesis Involving Hrp-Mediated Delivery of Avr-like Proteins into Plant Cells.

The Hrp secretion system of *P. syringae* is shown. The system is proposed to deliver some proteins (e.g., HrpZ) to the apoplast upon expression of the *hrp* regulon and others (e.g., Avr proteins) into plant cells upon receipt of a host contact signal. The latter transfer would require a type III pathway (purple structure) traversing both bacterial membranes and the plant cell wall (CW) and plasma membrane (PM). HrpZ in the apoplast may directly promote parasitism by eliciting the XR (K^+ efflux/H^+ influx). The Avr proteins may collectively promote parasitism (green panels) or individually trigger the HR (pink panels), as depicted. Many Avr proteins and host targets may be involved in these interactions (denoted by the triple dots). The model shows Avr proteins with long red arms denoting strong avirulence phenotypes and shorter green arms denoting weaker virulence phenotypes. However, it is possible that the same domains (or enzymatic products) are involved in both phenotypes and that some virulence proteins are not recognized by the *R* gene surveillance system.

(e.g., AvrBs2 with its possible agrocinopine synthase activity). The model suggests that pathogenesis may involve a distinct phase in which bacteria are attached to host cells. If so, this phase may be bracketed by entry and systemic spread phases in which diffusible virulence factors are more important.

The Hrp-delivered Avr protein model suggests that coevolutionary processes have led to the present genetics of pathogenicity in the necrogenic plant pathogens. The first coevolutionary parry may have been the Hrp delivery of a protein targeting the host metabolism for parasitic benefit. The host would be expected to counter with genetic changes that would reduce the responsiveness of the target to the parasite protein and deploy a new *R* gene such that the parasite protein triggers the HR. Both of these changes in the host would lead to the subsequent deployment by the pathogen of a new parasitic protein. Ultimately, reiteration of this process would lead to the evolution of bacteria that harbor a single, absolutely required Hrp system and a plethora of Hrp-delivered proteins that are collectively but not individually required for virulence but that individually can confer avirulence.

This scenario invites three fundamental questions. First, how does the apoplast have to be modified to support bacterial growth, and what metabolic processes in the interior of plant cells are targeted to this end? Second, do the virulence functions of Avr proteins target essentially universal plant constituents or does their specificity contribute to host specificity at the pathovar–species level? Third, how is Hrp-mediated pathogenicity integrated with the major virulence systems that produce the factors making these bacterial parasites so destructive to plants?

VIRULENCE FACTORS

Toxins and Extracellular Polysaccharides

The toxins produced by the necrogenic Gram-negative pathogens (primarily the *P. syringae* pathovars) differ in several ways from the Hrp-associated proteins discussed above. The toxins are secondary metabolites (mostly small peptides). They show no host specificity, typically do not contribute to bacterial multiplication in plants, and are highly diffusible, often producing characteristic symptoms spreading well beyond developed lesions (reviewed in Gross, 1991). The *P. syringae* toxins differ according to which pathovars produce them, their molecular targets, and their contribution to virulence (Table 2). Whereas some of the host-specific toxins of fungal pathogens are absolutely required for pathogenesis (see Walton, 1996, in this issue), bacterial toxins are generally considered to be virulence factors. They may contribute to the production of certain symptoms but in a manner that is not essential for pathogenesis.

The actual roles of individual toxins in pathogenesis are still unclear. Toxins are produced by some nonpathogenic strains of *P. syringae* (e.g., see Adetuyi et al., 1995), and many toxins also have antimicrobial activity and thus may function primarily to reduce microbial competition during epiphytic or pathogenic colonization (Gross, 1991). Furthermore, strains of *P. syringae* pv *tabaci* and *P. syringae* pv *coronafaciens* spontaneously delete tabtoxin biosynthetic genes in the field without losing pathogenicity (Willis et al., 1991). However, coronatine production contributes to the multiplication of *P. s. tomato* in plants, and dip-inoculation assays, which mimic field conditions, suggest that the toxin contributes to an early stage in pathogenesis by suppressing defense gene expression (Mittal and Davis, 1995).

Recent progress has focused on understanding the biosynthesis, regulation, and modes of action of toxins. For example, we now have evidence that syringomycin and coronatine are synthesized in part by nonribosomal thiotemplate mechanisms (Ullrich and Bender, 1994; Zhang et al., 1995), that host-associated phenolic glycosides and sugars (syringomycin) or low temperature (phaseolotoxin and coronatine) are important environmental factors sensed by bacterial regulators of toxin production (Mo and Gross, 1991; Rowley et al., 1993; Ullrich et al., 1995), and that syringomycin and coronatine may disrupt signaling within plant cells: syringomycin by opening calcium channels (Takemoto, 1992; Hutchison et al., 1995) and coronatine by mimicking, at least partially, methyl jasmonate, which regulates wound-inducible defense responses (Feys et al., 1994; Weiler et al., 1994; Palmer and Bender, 1995).

EPSs, unlike toxins, are produced by most bacteria, including many plant pathogens, and are secreted as a loose slime or as capsular material. EPSs are thought to protect free-living bacteria from a variety of environmental stresses and may aid pathogenesis by sustaining water-soaking of intercellular spaces, altering the accessibility of antimicrobial compounds or defense-activating signals, and blocking the xylem and thereby producing wilt symptoms (reviewed in Denny, 1995). EPS production, particularly its role in pathogenesis as determined through transposon mutagenesis, has been explored most extensively in *R. solanacearum* (Denny and Baek, 1991; Kao et al., 1992), *E. amylovora* (Steinberger and Beer, 1988; Bernhard et al., 1993), and *E. stewartii* (Coplin and Majerczak, 1990). EPS is generally a virulence factor in these bacteria, contributing to wilt and water-soaking symptoms without be-

Table 2. Toxins Produced by *P. syringae* Pathovars[a]

Toxin	Pathovar	Structure Type	Function or Target	Symptoms
Syringomycin	*syringae*	Cyclic lipodepsipeptide	Forms pores in plasma membrane	Necrosis
Syringopeptin	*syringae*	Cyclic lipodepsipeptide	Forms pores in plasma membrane	Necrosis
Coronatine	*tomato*, *glycinea*, others	Polyketide and cyclized amino acid	Molecular mimic of plant signal molecule methyl jasmonate	Chlorosis
Tagetitoxin	*tagetis*	Hemithioketal	Inhibitor of chloroplast RNA polymerase	Chlorosis
Phaseolotoxin	*phaseolicola*	Sulpho-diamino-phosphinyl tripeptide	Inhibitor of ornithine carbamoyltransferase	Chlorosis
Tabtoxin	*tabaci* and several others	β-lactam–containing dipeptide	Inhibitor of glutamine synthetase	Chlorosis

[a] All references are given in the text or in Gross (1991), except for those for tagetitoxin action; see Mathews and Durbin (1990).

ing absolutely required for pathogenesis and without any discernible host specificity (unlike the EPSs of *Rhizobium* spp; Leigh and Walker, 1994). Thus, various toxins and EPSs may contribute to pathogenesis in different ways with respect to mechanism and importance. Better characterization of the regulation and genetics of biosynthesis of toxins and EPS is providing the foundation for a rigorous analysis of their role in multifactorial pathogenesis.

Pectic Enzymes

The bacterial soft rots caused by the necrotrophic pathogens *E. carotovora*, *E. chrysanthemi*, and *P. viridiflava* differ substantially from the diseases caused by the biotrophic pathogens that have been considered up to this point (Table 1). These bacteria have a wide host range, particularly among plants with fleshy parenchymatous tissues, disease incidence is more dependent on environmental conditions that compromise the host, and pathogenesis is dominated by pectic enzymes that cleave α-1,4-galacturonsyl linkages in plant cell wall polymers by hydrolysis (polygalacturonases) or β-elimination (pectate or pectin lyases; reviewed in Perombelon and Kelman, 1980; Barras et al., 1994). Because of the structural importance and unique accessibility of pectic polymers in the primary cell walls and middle lamellae of dicots and some monocots, pectic enzymes are the big guns of the brute-force approach to pathogenesis, and they cause both cell killing and tissue maceration, the primary symptoms of soft rot disease (Collmer and Keen, 1986).

P. viridiflava secretes a single pectate lyase (Pel), which is required for its opportunistic pathogenicity in market vegetables (Liao et al., 1988). Conversely, the more widespread and versatile pathogens *E. carotovora* and *E. chrysanthemi* secrete complexes of pectic enzymes dominated by multiple Pel isozymes (Barras et al., 1994). All of these enzymes (except the SOS-inducible pectin lyases) are secreted via the type II pathway, and bacterial secretion mutants are unable to cause soft rots (Barras et al., 1994). However, *E. chrysanthemi* mutants lacking all five of the "major," pectate-inducible Pel isozymes retain partial maceration virulence and can be seen to produce a second set of Pel isozymes (Beaulieu et al., 1993; Kelemu and Collmer, 1993; Alfano et al., 1995; Lojkowska et al., 1995). Interestingly, individual *pel* mutations reveal that the respective Pel isozymes vary in importance in different hosts and may collectively contribute to the wide host range of *E. chrysanthemi* (Beaulieu et al., 1993). The *E. chrysanthemi* Pel isozymes also differ in their relative contribution to maceration and systemic invasion of plants (Barras et al., 1987; Boccara et al., 1988) and in their regulation by pectate-derived inducers, temperature, nitrogen starvation, oxygen levels, iron concentration, and growth phase, as controlled by at least three regulatory proteins—KdgR, PecS, and PecT (reviewed in Hugouvieux-Cotte-Pattat et al., 1996). Thus, a particularly firm molecular genetic foundation is being built for ultimately understanding how the proliferation and regulation of pectic enzymes have supported the evolution of pathogenicity based on disassembly of the plant cell wall.

Bacterial Cell-Cell Signaling and Global Regulation of Virulence

The development of substantial pathogen populations is often an important factor in the diseases caused by necrogenic Gram-negative bacteria (Perombelon and Kelman, 1980; Hirano and Upper, 1990), and there is now evidence that at least two of these bacteria, *E. carotovora* (Jones et al., 1993; Pirhonen et al., 1993) and *E. stewartii* (Beck von Bodman and Farrand, 1995), use cell–cell signaling for "quorum sensing" regulation of virulence gene expression (Fuqua et al., 1993). Quorum sensing was first described in the marine symbiont *Vibrio fisheri*, in which an *N*-acyl homoserine lactone "autoinducer" regulates the expression of *lux* (bioluminescence) genes in a cell density–dependent manner: a threshold level of the autoinducer, which is a diffusible product of LuxI, signals a quorum population and the induction of LuxR-regulated genes (Fuqua et al., 1996). This phenomenon was considered unique to bioluminescence regulation until the discovery of autoinducers and LuxI/R homologs in plant pathogens and subsequently in a variety of other bacteria (Salmond et al., 1995).

Although quorum sensing seems too polite a term for the "mob" attack of *E. carotovora*, it explains a puzzling aspect of the action of pectic enzymes in pathogenesis. That is, individual pectic enzymes, their products (especially dodecauronates), or pectolytic culture fluids from *E. carotovora* can elicit plant defenses that protect plants from bacteria (Hahn et al., 1988; Yang et al., 1992; Palva et al., 1993). The discovery that *E. carotovora* mutants lacking the *luxI* homolog fail to produce high levels of pectic enzymes or cause soft-rot disease unless exogenously supplied with an autoinducer suggests that the pathogen withholds pectolytic attack until it has a "mob" large enough to overwhelm the host defenses that it triggers (Jones et al., 1993; Pirhonen et al., 1993). Such a parasitic strategy may fundamentally distinguish necrotrophs like *E. carotovora* from biotrophs like *P. syringae* (Collmer and Bauer, 1994).

Quorum sensing may also play an important role in bacterial survival in the rhizosphere. For example, the biological control agent *P. aureofaciens* 30-84 uses an autoinducer and LuxI/R homologs to regulate production of phenazine antibiotics required for effective suppression of fungal pathogens and full bacterial fitness in the soil (Mazzola et al., 1992; Pierson et al., 1994). It has been postulated that plant nutrients released by pathogen attack allow an increase in the *P. aureofaciens* population and autoinduction such that the antibiotics are produced precisely when demanded by the presence of a food source and a competitor (Pierson and Pierson, 1996). Moreover, because many autoinducers isolated from different bacteria have identical structures, it is possible that interbacterial quorum sensing or cross-talk is a normal part of life in the

rhizosphere (Pierson et al., 1994; Fuqua et al., 1996; see also Handelsman and Stabb, 1996, in this issue).

The LuxI/R homologs in *E. carotovora*, like the AraC homologs and ECF sigma factors discussed above, are representative of several global regulatory factors that have been recruited to control the expression of multiple virulence genes. Similarly, in *P. syringae*, the two-component family regulatory proteins LemA and GacA control production of syringomycin, extracellular protease, and lesion development (Hrabak and Willis, 1992, 1993; Rich et al., 1994); in *R. solanacearum*, a hierarchy of several regulatory proteins and a volatile factor, which may function analogously to an autoinducer, regulates virulence and the production of EPS and several extracellular proteins (Clough et al., 1994; J. Huang et al., 1995); in *X. c. campestris*, a similar subset of virulence factors is coordinately regulated by several independent regulatory genes (Dow and Daniels, 1994); and in *E. carotovora*, *rsmA* mutants reveal the autoinduction system itself to be part of a larger regulon (Cui et al., 1995). The challenge now is to understand the cues that enable these regulatory networks to orchestrate the intrinsically dynamic and multifactorial process of bacterial pathogenesis.

EXPLORING BACTERIAL PATHOGENESIS IN PLANTS FROM A NEW PERSPECTIVE

We are still seeking answers to the most fundamental questions regarding the mechanisms by which necrogenic bacterial pathogens colonize the apoplast and produce plant diseases or how these diseases might be effectively controlled. However, new perspectives on these problems reveal where and how to look for their answers in the future. First, we now see that plant and animal pathogens have some features that may be generally common to bacterial parasites of higher eukaryotes. These include virulence-associated mechanisms for obtaining iron (Expert et al., 1996), conserved systems for deploying virulence proteins (Van Gijsegem et al., 1993), and convergent pathogenic strategies (Collmer and Bauer, 1994). Recent reports that *P. aeruginosa* strain UCBPP-PA14 requires common virulence factors for its opportunistic attack on both mouse and Arabidopsis and that the *X. c. vesicatoria* AvrRxv and *Y. pseudotuberculosis* YopJ proteins show sequence similarity further support the promise of a more global approach to pathogenesis research (Rahme et al., 1995; Leach and White, 1996).

We also now see that the molecules deployed by pathogens to interact directly with the host typically contribute only quantitatively to virulence. Thus, previous virulence mutant screens have given us an incomplete inventory dominated by mutants with pleiotropic regulatory and secretion phenotypes. Promising approaches for finding genes with subtle virulence phenotypes include more sensitive assays for reduced fitness in plants, analysis of DNA sequences in pathogenicity islands, better assays for protein traffic through pathways associated with virulence, and the identification of genes expressed during pathogenesis (e.g., Osbourn et al., 1987). Obtaining the complete inventory of proteins traveling the Hrp pathway is particularly important: because Hrp secretion mutants typically lose all parasitic ability, these proteins collectively must make the key modifications to plant metabolism that are required for bacterial growth in the apoplast. Understanding what these proteins do should reveal why the apoplast is so inhospitable to the vast majority of bacteria.

Finally, we now see that bacterium–plant interactions are highly coevolved and dynamic processes at the molecular, cellular, and colony–tissue level. For example, Avr proteins, which appear to promote the most insidious form of parasitism, also trigger the most potent defense responses; because of the likely contact-dependent operation of the Hrp secretion system, intimate cell–cell interactions are almost certainly critical in pathogenesis; and the interplay of attack-promoting signaling between bacteria and defense-promoting signaling between host cells may be crucial in the development of many diseases. For us to better understand these interactions, future research must expand beyond molecular genetics to include more biochemistry and cell biology. A full understanding may require cell biological approaches capable of monitoring ensembles of virulence and defense systems in interacting populations of pathogen and host cells. For plant biologists studying these parasites that live up against the wall of plant cells, perhaps the ultimate questions are how has their intimate reach shaped the evolution of modern plants and what new tools for exploring plant biology might they yield?

ACKNOWLEDGMENTS

Work in our laboratory was supported in part by National Science Foundation Grant No. MCB 9305178 and National Research Initiative Competitive Grants Program/U.S. Department of Agriculture Grant No. 94-37303-0734.

REFERENCES

Adetuyi, F.C., Isogai, A., Di Giorgio, D., Ballio, A., and Takemoto, J.Y. (1995). Saprophytic *Pseudomonas syringae* strain M1 of wheat produces cyclic lipodepsipeptides. FEMS Microbiol. Lett. **131,** 63–67.

Alfano, J.R., Ham, J.H., and Collmer, A. (1995). Use of Tn*5*tac1 to clone a *pel* gene encoding a highly alkaline, asparagine-rich pectate lyase isozyme from an *Erwinia chrysanthemi* mutant with deletions affecting the major pectate lyase isozymes. J. Bacteriol. **177,** 4553–4556.

Alfano, J.R., Bauer, D.W., Milos, T.M., and Collmer, A. (1996). Analysis of the role of the *Pseudomonas syringae* pv. *syringae* HrpZ harpin in elicitation of the hypersensitive response in tobacco using functionally nonpolar deletion mutations, truncated HrpZ fragments, and *hrmA* mutations. Mol. Microbiol. **19,** 715–728.

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

Arlat, M., Van Gijsegem, F., Huet, J.C., Pernollet, J.C., and Boucher, C.A. (1994). PopA1, a protein which induces a hypersensitive-like response on specific *Petunia* genotypes, is secreted via the Hrp pathway of *Pseudomonas solanacearum.* EMBO J. **13,** 543–553.

Atkinson, M.M. (1993). Molecular mechanisms of pathogen recognition by plants. In Advances in Plant Pathology, Vol. 10, J.H. Andrews and I.C. Tommerup, eds (New York: Academic Press), pp. 35–64.

Atkinson, M.M., and Baker, C.J. (1987a). Alteration of plasmalemma sucrose transport in *Phaseolus vulgaris* by *Pseudomonas syringae* pv. *syringae* and its association with K^+/H^+ exchange. Phytopathology **77,** 1573–1578.

Atkinson, M.M., and Baker, C.J. (1987b). Association of host plasma membrane K^+/H^+ exchange with multiplication of *Pseudomonas syringae* pv. *syringae* in *Phaseolus vulgaris.* Phytopathology **77,** 1273–1279.

Baker, C.J., and Orlandi, E.W. (1995). Active oxygen in plant pathogenesis. Annu. Rev. Phytopathol. **33,** 299–321.

Barinaga, M. (1996). A shared strategy for virulence. Science **272,** 1261–1263.

Barny, M.-A. (1995). *Erwinia amylovora hrpN* mutants, blocked in harpin synthesis, express a reduced virulence on host plants and elicit variable hypersensitive reactions on tobacco. Eur. J. Plant Pathol. **101,** 333–340.

Barras, F., Thurn, K.K., and Chatterjee, A.K. (1987). Resolution of four pectate lyase structural genes of *Erwinia chrysanthemi* (EC16) and characterization of the enzymes produced in *Escherichia coli.* Mol. Gen. Genet. **209,** 319–325.

Barras, F., Van Gijsegem, F., and Chatterjee, A.K. (1994). Extracellular enzymes and pathogenesis of soft-rot *Erwinia.* Annu. Rev. Phytopathol. **32,** 201–234.

Bauer, D.W., Bogdanove, A.J., Beer, S.V., and Collmer, A. (1994). *Erwinia chrysanthemi hrp* genes and their involvement in soft rot pathogenesis and elicitation of the hypersensitive response. Mol. Plant-Microbe Interact. **7,** 573–581.

Bauer, D.W., Wei, Z.-M., Beer, S.V., and Collmer, A. (1995). *Erwinia chrysanthemi* $harpin_{Ech}$: An elicitor of the hypersensitive response that contributes to soft-rot pathogenesis. Mol. Plant-Microbe Interact. **8,** 484–491.

Beaulieu, C., Boccara, M., and Van Gijsegem, F. (1993). Pathogenic behavior of pectinase-defective *Erwinia chrysanthemi* mutants on different plants. Mol. Plant-Microbe Interact. **6,** 197–202.

Beck von Bodman, S., and Farrand, S.K. (1995). Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an *N*-acylhomoserine lactone autoinducer. J. Bacteriol. **177,** 5000–5008.

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, H. Hennecke and D.P.S. Verma, eds (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 53–60.

Bent, A.F. (1996). Plant disease resistance genes: Function meets structure. Plant Cell **8,** 1757–1771.

Bent, A.F., Kunkel, B.N., Dahlbeck, D., Brown, K.L., Schmidt, R., Giraudat, J., Leung, J., and Staskawicz, B.J. (1994). *RPS2* of *Arabidopsis thaliana:* A leucine-rich repeat class of plant disease resistance genes. Science **265,** 1856–1860.

Bernhard, T., Coplin, D.L., and Geider, K. (1993). A gene cluster for amylovoran synthesis in *Erwinia amylovora*: Characterization and relation to *cps* genes in *Erwinia stewartii.* Mol. Gen. Genet. **239,** 158–168.

Bisgrove, S.R., Simonich, M.T., Smith, N.M., Sattler, A., and Innes, R.W. (1994). A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. Plant Cell **6,** 927–933.

Boccara, M., Diolez, A., Rouve, M., and Kotoujansky, A. (1988). The role of individual pectate lyases of *Erwinia chrysanthemi* strain 3937 in pathogenicity on *saintpaulia* plants. Physiol. Mol. Plant Pathol. **33,** 95–104.

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., 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* spp. J. Bacteriol. **178,** 1720–1730.

Bonas, U. (1994). *hrp* genes of phytopathogenic bacteria. In Current Topics in Microbiology and Immunology, Vol. 192: Bacterial Pathogenesis of Plants and Animals—Molecular and Cellular Mechanisms, J.L. Dangl, ed (Berlin: Springer-Verlag), pp. 79–98.

Brown, I., Mansfield, J., Irlam, I., Conrads-Strauch, J., and Bonas, U. (1993). Ultrastructure of interactions between *Xanthomonas campestris* pv. *vesicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the AvrBs3 protein. Mol. Plant-Microbe Interact. **6,** 376–386.

Clough, S.J., Schell, M.A., and Denny, T.P. (1994). Evidence for involvement of a volatile extracellular factor in *Pseudomonas solanacearum* virulence gene expression. Mol. Plant-Microbe Interact. **7,** 621–630.

Collmer, A., and Bauer, D.W. (1994). *Erwinia chrysanthemi* and *Pseudomonas syringae*: Plant pathogens trafficking in virulence proteins. In Current Topics in Microbiology and Immunology, Vol. 192: Bacterial Pathogenesis of Plants and Animals—Molecular and Cellular Mechanisms, J.L. Dangl, ed (Berlin: Springer-Verlag), pp. 43–78.

Collmer, A., and Keen, N.T. (1986). The role of pectic enzymes in plant pathogenesis. Annu. Rev. Phytopathol. **24,** 383–409.

Collmer, A., Bauer, D.W., Alfano, J.R., Preston, G., Loniello, A.O., Huang, H.-C., and He, S.Y. (1994). The role of *Pseudomonas syringae* and *Erwinia chrysanthemi hrp* gene products in plant interactions. In Advances in Molecular Genetics of Plant–Microbe Interactions, Vol. 3, M.J. Daniels, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 49–56.

Coplin, D.L., and Majerczak, D.R. (1990). Extracellular polysaccharide genes in *Erwinia stewartii*: Directed mutagenesis and complementation analysis. Mol. Plant-Microbe Interact. **3,** 286–292.

Crute, I.R., and Pink, D.A.C. (1996). Genetics and utilization of pathogen resistance in plants. Plant Cell **8,** 1747–1755.

Cui, Y., Chatterjee, A., Liu, Y., Dumenyo, C.K., and Chatterjee, A.K. (1995). Identification of a global repressor gene, *rsmA*, of *Erwinia*

carotovora subsp. *carotovora* that controls extracellular enzymes, *N*-(3-oxohexanoyl)-L-homoserine lactone, and pathogenicity in soft-rotting *Erwinia* spp. J. Bacteriol. **177,** 5108–5115.

Dangl, J.L. (1994). The enigmatic avirulence genes of phytopathogenic bacteria. In Current Topics in Microbiology and Immunology, Vol. 192: Bacterial Pathogenesis of Plants and Animals—Molecular and Cellular Mechanisms, J.L. Dangl, ed (Berlin: Springer-Verlag), pp. 99–118.

Dangl, J.L., Dietrich, R.A., and Richberg, M.H. (1996). Death don't have no mercy: Cell death programs in plant–microbe interactions. Plant Cell **8,** 1793–1807.

Denny, T.P. (1995). Involvement of bacterial polysaccharide in plant pathogenesis. Annu. Rev. Phytopathol. **33,** 173–197.

Denny, T.P., and Baek, S.-R. (1991). Genetic evidence that extracellular polysaccharide is a virulence factor of *Pseudomonas solanacearum.* Mol. Plant-Microbe Interact. **4,** 198–206.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A., and Dangl, J.L. (1994). Arabidopsis mutants simulating disease resistance response. Cell **77,** 565–577.

Dow, J.M., and Daniels, M.J. (1994). Pathogenicity determinants and global regulation of pathogenicity of *Xanthomonas campestris* pv. *campestris.* In Current Topics in Microbiology and Immunology, Vol. 192: Bacterial Pathogenesis of Plants and Animals—Molecular and Cellular Mechanisms, J.L. Dangl, ed (Berlin: Springer-Verlag), pp. 29–41.

Expert, D., Enard, C., and Masclaux, C. (1996). The role of iron in plant host–pathogen interactions. Trends Microbiol. **4,** 232–237.

Fenselau, S., and Bonas, U. (1995). Sequence and expression analysis of the *hrpB* pathogenicity locus 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.

Fenselau, S., Balbo, I., and Bonas, U. (1992). Determinants of pathogenicity in *Xanthomonas campestris* pv. *vesicatoria* are related to proteins involved in secretion in bacterial pathogens of animals. Mol. Plant-Microbe Interact. **5,** 390–396.

Feys, B.J.F., Benedetti, C.E., Penfold, C.N., and Turner, J.G. (1994). Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Mol. Plant-Microbe Interact. **6,** 751–759.

Flor, H.H. (1956). The complementary genic systems in flax and flax rust. Adv. Genet. **8,** 29–54.

Fuqua, W.C., Winans, S.C., and Greenberg, E.P. (1993). Quorum sensing in bacteria: The LuxR/LuxI family of cell density responsive transcriptional regulators. J. Bacteriol. **176,** 269–275.

Fuqua, W.C., Winans, S.C., and Greenberg, E.P. (1996). Census and consensus in bacterial ecosystems: The LuxR–LuxI family of quorum-sensing transcriptional regulators. Annu. Rev. Microbiol. **50,** 727–751.

Galan, J.E. (1996). Molecular genetic bases of *Salmonella* entry into host cells. Mol. Microbiol. **20,** 263–271.

Genin, S., Gough, C.L., Zischek, C., and Boucher, C.A. (1992). Evidence that the *hrpB* gene encodes a positive regulator of pathogenicity genes from *Pseudomonas solanacearum.* Mol. Microbiol. **6,** 3065–3076.

Glazebrook, J., and Ausubel, F.M. (1994). Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. Proc. Natl. Acad. Sci. USA **91,** 8955–8959.

Glazener, J.A., Orlandi, E.W., and Baker, C.J. (1996). The active oxygen response of cell suspensions to incompatible bacteria is not sufficient to cause hypersensitive cell death. Plant Physiol. **110,** 759–763.

Goodman, R.N., and Novacky, A.J. (1994). The Hypersensitive Reaction of Plants to Pathogens: A Resistance Phenomenon. (St. Paul, MN: APS Press).

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.

Grant, M.R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R.W., and Dangl, J.L. (1995). Structure of the Arabidopsis *RPM1* gene enabling dual specificity disease resistance. Science **269,** 843–846.

Greenberg, J.T., Guo, A., Klessig, D.F., and Ausubel, F.M. (1994). Programmed cell death in plants: A pathogen-triggered response activated coordinately with multiple defense functions. Cell **77,** 551–563.

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.

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.

Groisman, E.A., and Ochman, H. (1993). Cognate gene clusters govern invasion of host epithelial cells by *Salmonella typhimurium* and *Shigella flexneri.* EMBO J. **12,** 3779–3787.

Gross, D.C. (1991). Molecular and genetic analysis of toxin production by pathovars of *Pseudomonas syringae.* Annu. Rev. Phytopathol. **29,** 247–278.

Hahn, M.G., Bucheli, P., Cervone, F., Doares, S.H., O'Neill, R.A., Darvill, A., and Albersheim, P. (1988). The roles of cell wall constituents in plant–pathogen interactions. In Plant–Microbe Interactions: Molecular and Genetic Perspectives, Vol. 3, E. Nester and T. Kosuge, eds (New York: Macmillan), pp. 131–181.

Hammond-Kosack, K.E., and Jones, J.D.G. (1996). Resistance gene–dependent plant defense responses. Plant Cell **8,** 1773–1791.

Handelsman, J., and Stabb, E.V. (1996). Biocontrol of soilborne plant pathogens. Plant Cell **8,** 1855–1869.

He, S.Y., Huang, H.-C., and Collmer, A. (1993). *Pseudomonas syringae* pv. *syringae* harpin$_{Pss}$: A protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell **73,** 1255–1266.

He, S.Y., Bauer, D.W., Collmer, A., and Beer, S.V. (1994). Hypersensitive response elicited by *Erwinia amylovora* harpin requires active plant metabolism. Mol. Plant-Microbe Interact. **7,** 289–292.

Heu, S., and Hutcheson, S.W. (1993). Nucleotide sequence and properties of the *hrmA* locus associated with the *Pseudomonas syringae*

pv. *syringae* 61 *hrp* gene cluster. Mol. Plant-Microbe Interact. **6,** 553–564.

Hirano, S.S., and Upper, C.D. (1990). Population biology and epidemiology of *Pseudomonas syringae.* Annu. Rev. Phytopathol. **28,** 155–177.

Holliday, M.J., Keen, N.T., and Long, M. (1981). Cell death patterns and accumulation of fluorescent material in the hypersensitive response of soybean leaves to *Pseudomonas syringae* pv. *glycinea.* Physiol. Plant Pathol. **18,** 279–287.

Hrabak, E.M., and Willis, D.K. (1992). The *lemA* gene required for pathogenicity of *Pseudomonas syringae* pv. *syringae* on bean is a member of a family of two-component regulators. J. Bacteriol. **174,** 3011–3020.

Hrabak, E.M., and Willis, D.K. (1993). Involvement of the *lemA* gene in production of syringomycin and protease by *Pseudomonas syringae* pv. *syringae.* Mol. Plant-Microbe Interact. **6,** 368–375.

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

Huang, H.-C., Hutcheson, S.W., and Collmer, A. (1991). Characterization of the *hrp* cluster from *Pseudomonas syringae* pv. *syringae* 61 and Tn*phoA* tagging of genes encoding exported or membrane-spanning Hrp proteins. Mol. Plant-Microbe Interact. **4,** 469–476.

Huang, H.-C., He, S.Y., Bauer, D.W., and Collmer, A. (1992). The *Pseudomonas syringae* pv. *syringae* 61 *hrpH* product: An envelope protein required for elicitation of the hypersensitive response in plants. J. Bacteriol. **174,** 6878–6885.

Huang, H.-C., Xiao, Y., Lin, R.-H., Lu, Y., Hutcheson, S.W., and Collmer, A. (1993). Characterization of the *Pseudomonas syringae* pv. *syringae hrpJ* and *hrpI* genes: Homology of HrpI to a superfamily of proteins associated with protein translocation. Mol. Plant-Microbe Interact. **6,** 515–520.

Huang, H.-C., Lin, R.-W., Chang, C.-J., Collmer, A., and Deng, W.-L. (1995). The complete *hrp* gene cluster of *Pseudomonas syringae* pv. *syringae* 61 includes two blocks of genes required for harpin$_{Pss}$ secretion that are arranged colinearly with *Yersinia ysc* homologs. Mol. Plant-Microbe Interact. **8,** 733–746.

Huang, J., Carney, B.F., Denny, T.P., Weissinger, A.K., and Schell, M.A. (1995). A complex network regulates expression of *eps* and other virulence genes in *Pseudomonas solanacearum.* J. Bacteriol. **177,** 1259–1267.

Hugouvieux-Cotte-Pattat, N., Condemine, G., Nasser, W., and Reverchon, S. (1996). Regulation of pectinolysis in *Erwinia chrysanthemi.* Annu. Rev. Microbiol. **50,** 213–257.

Hutchison, M.L., Tester, M.A., and Gross, D.C. (1995). Role of biosurfactant and ion channel-forming activities of syringomycin in transmembrane ion flux: A model for the mechanism of action in the plant–pathogen interaction. Mol. Plant-Microbe Interact. **8,** 610–620.

Huynh, T.V., Dahlbeck, D., and Staskawicz, B.J. (1989). Bacterial blight of soybean: Regulation of a pathogen gene determining host cultivar specificity. Science **245,** 1374–1377.

Innes, R.W., Bent, A.F., Kunkel, B.N., Bisgrove, S.R., and Staskawicz, B.J. (1993). Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. J. Bacteriol. **175,** 4859–4869.

Jakobek, J.L., and Lindgren, P.B. (1993). Generalized induction of defense responses in bean is not correlated with the induction of the hypersensitive reaction. Plant Cell **5,** 49–56.

Jakobek, J.L., Smith, J.A., and Lindgren, P.B. (1993). Suppression of bean defense responses by *Pseudomonas syringae.* Plant Cell **5,** 57–63.

Jones, S., Yu, B., Bainton, N.J., Birdsall, M., Bycroft, B.W., Chhabra, S.R., Cox, A.J.R., Golby, P., Reeves, P.J., Stephens, S., Winson, M.K., Salmond, G.P.C., Stewart, G.S.A.B., and Williams, P. (1993). The *lux* autoinducer regulates the production of exoenzyme virulence determinants in *Erwinia carotovora* and *Pseudomonas aeruginosa.* EMBO J. **12,** 2477–2482.

Kang, Y., Huang, J., Guozhang, M., He, L.-Y., and Schell, M.A. (1994). Dramatically reduced virulence of mutants of *Pseudomonas solanacearum* defective in export of extracellular proteins across the outer membrane. Mol. Plant-Microbe Interact. **7,** 370–377.

Kao, C.C., Barlow, E., and Sequeira, L. (1992). Extracellular polysaccharide is required for wild-type virulence of *Pseudomonas solacearum.* J. Bacteriol. **174,** 1068–1071.

Keen, N.T. (1990). Gene-for-gene complementarity in plant–pathogen interactions. Annu. Rev. Genet. **24,** 447–463.

Keen, N.T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D., and Staskawicz, B. (1990). Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant-Microbe Interact. **3,** 112–121.

Kelemu, S., and Collmer, A. (1993). *Erwinia chrysanthemi* EC16 produces a second set of plant-inducible pectate lyase isozymes. Appl. Environ. Microbiol. **59,** 1756–1761.

Klement, Z. (1963). Rapid detection of pathogenicity of phytopathogenic pseudomonads. Nature **199,** 299–300.

Klement, Z. (1982). Hypersensitivity. In Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, eds (New York: Academic Press), pp. 149–177.

Klement, Z., Farkas, G.L., and Lovrekovich, L. (1964). Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology **54,** 474–477.

Kobayshi, D.Y., Tamaki, S.J., and Keen, N.T. (1989). Cloned avirulence genes from the tomato pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. Proc. Natl. Acad. Sci. USA **86,** 157–161.

Leach, J.E., and White, F.F. (1996). Bacterial avirulence genes. Annu. Rev. Phytopathol. **34,** 153–179.

Leigh, J.A., and Walker, G.C. (1994). Exopolysaccharides of *Rhizobium*: Synthesis, regulation and symbiotic function. Trends Genet. **10,** 63–67.

Levine, A., Tenhaken, R., Dixon, R., and Lamb, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79,** 583–593.

Liao, C.-H., Hung, H.Y., and Chatterjee, A.K. (1988). An extracellular pectate lyase is the pathogenicity factor of the soft-rotting bacterium *Pseudomonas viridiflava.* Mol. Plant-Microbe Interact. **1,** 199–206.

Lidell, M.C., and Hutcheson, S.W. (1994). Characterization of the *hrpJ* and *hrpU* operons of *Pseudomonas syringae* pv. *syringae* Pss61: Similarity with components of enteric bacteria involved in flagellar biogenesis and demonstration of their role in harpin$_{Pss}$ secretion. Mol. Plant-Microbe Interact. **7,** 488–497.

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

Lojkowska, E., Masclaux, C., Boccara, M., Robert-Baudouy, J., and Hugouvieux-Cotte-Pattat, N. (1995). Characterization of the *pelL* gene encoding a novel pectate lyase of *Erwinia chrysanthemi* 3937. Mol. Microbiol. **16,** 1183–1195.

Long, M., Barton-Willis, P., Staskawicz, B.J., Dahlbeck, D., and Keen, N.T. (1985). Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to *Pseudomonas syringae* pv. *glycinea*. Phytopathology **75,** 235–239.

Long, S.R. (1996). *Rhizobium* symbiosis: Nod factors in perspective. Plant Cell **8,** 1885–1898.

Lorang, J.M., and Keen, N.T. (1995). Characterization of *avrE* from *Pseudomonas syringae* pv. *tomato*: A *hrp*-linked avirulence locus consisting of at least two transcriptional units. Mol. Plant-Microbe Interact. **8,** 49–57.

Lorang, J.M., Shen, H., Kobayashi, D., Cooksey, D., and Keen, N.T. (1994). *avrA* and *avrE* in *Pseudomonas syringae* pv. *tomato* PT23 play a role in virulence on tomato plants. Mol. Plant-Microbe Interact. **7,** 508–515.

Macnab, R.M. (1996). Flagella and motility. In *Escherichia coli* and *Salmonella*, Vol. 1, F.C. Neidhardt, ed (Washington, DC: ASM Press), pp. 123–145.

Mansfield, J., Jenner, C., Hockenhull, R., Bennett, M.A., and Stewart, R. (1994). Characterization of *avrPphE*, a gene for cultivar-specific avirulence from *Pseudomonas syringae* pv. *phaseolicola* which is physically linked to *hrpY*, a new *hrp* gene identified in the halo-blight bacterium. Mol. Plant-Microbe Interact. **7,** 726–739.

Mathews, D.E., and Durbin, R.D. (1990). Tagetitoxin inhibits RNA synthesis directed by RNA polymerases from chloroplasts and *Escherichia coli*. J. Biol. Chem. **265,** 493–498.

Mazzola, M., Cook, R.J., Thomashow, L.S., Weller, D.M., and Pierson III, L.S. (1992). Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. Appl. Environ. Microbiol. **58,** 2616–2624.

Meier, B.M., and Slusarenko, A.J. (1993). Spatial and temporal accumulation of defense gene transcripts in bean (*Phaseolus vulgaris*) leaves in relation to bacteria-induced hypersensitive cell death. Mol. Plant-Microbe Interact. **6,** 453–466.

Midland, S.L., Keen, N.T., Sims, J.J., Midland, M.M., Stayton, M.M., Burton, V., Smith, M.J., Mazzola, E.P., Graham, K.J., and Clardy, J. (1993). The structures of syringolides 1 and 2: Novel C glycosidic elicitors from *Pseudomonas syringae* pv. *tomato*. J. Org. Chem. **58,** 2940–2945.

Mindrinos, M., Katagiri, F., Yu, G.-L., and Ausubel, F.M. (1994). The A. thaliana disease resistance gene *RPS2* encodes a protein containing a nucleotide-binding site and leucine-rich repeats. Cell **78,** 1089–1099.

Mittal, S., and Davis, K.R. (1995). Role of the phytotoxin coronatine in the infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*. Mol. Plant-Microbe Interact. **8,** 165–171.

Mo, Y.-Y., and Gross, D.C. (1991). Plant signal molecules activate the *syrB* gene, which is required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. J. Bacteriol. **173,** 5784–5792.

Niepold, F., Anderson, D., and Mills, D. (1985). Cloning determinants of pathogenesis from *Pseudomonas syringae* pathovar *syringae*. Proc. Natl. Acad. Sci. USA **82,** 406–410.

Oku, T., Alvarez, A.M., and Kado, C.I. (1995). Conservation of the hypersensitivity-pathogenicity regulatory gene *hrpX* of *Xanthomonas campestris* and *X. oryzae*. DNA Sequence **5,** 245–249.

Osbourn, A.E., Barber, C.E., and Daniels, M.J. (1987). Identification of plant-induced genes of the bacterial pathogen *Xanthomonas campestris* pathovar *campestris* using a promoter–probe plasmid. EMBO J. **6,** 23–28.

Palmer, D.A., and Bender, C.L. (1995). Ultrastructure of tomato leaf tissue treated with the Pseudomonad phytotoxin coronatine and comparison with methyl jasmonate. Mol. Plant-Microbe Interact. **8,** 683–692.

Palva, T.K., Holmstrom, K.-O., Heino, P., and Palva, E.T. (1993). Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. Mol. Plant-Microbe Interact. **6,** 190–196.

Perombelon, M.C.M., and Kelman, A. (1980). Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. **18,** 361–387.

Pierce, M., and Essenberg, M. (1987). Localization of phytoalexins in fluorescent mesophyll cells isolated from bacterial blight-infected cotton cotyledons and separated from other cells by fluorescence-activated cell sorting. Physiol. Mol. Plant Pathol. **31,** 273–290.

Pierson III, L.S., and Pierson, E.A. (1996). Phenazine antibiotic production in *Pseudomonas aureofaciens*: Role in rhizosphere ecology and pathogen suppression. FEMS Microbiol. Lett. **136,** 101–108.

Pierson III, L.S., Keppenne, V.D., and Wood, D.W. (1994). Phenazine antibiotic biosynthesis in *Pseudomonas aureofaciens* 30-84 is regulated by PhzR in response to cell density. J. Bacteriol. **176,** 3966–3974.

Pirhonen, M., Flego, D., Heikinheimo, R., and Palva, E.T. (1993). A small diffusible signal molecule is responsible for the global control of virulence and exoenzyme production in the plant pathogen *Erwinia carotovora*. EMBO J. **12,** 2467–2476.

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). Phenotypic 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.

Preston, G., Huang, H.-C., He, S.Y., and Collmer, A. (1995). The HrpZ proteins of *Pseudomonas syringae* pvs. *syringae, glycinea*, and *tomato* are encoded by an operon containing *Yersinia ysc* homologs and elicit the hypersensitive response in tomato but not soybean. Mol. Plant-Microbe Interact. **8,** 717–732.

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.

Rahme, L.G., Stevens, E.J., Wolfort, S.F., Shao, J., Tompkins, R.G., and Ausubel, F.M. (1995). Common virulence factors for bacterial pathogenicity in plants and animals. Science **268,** 1899–1902.

Reuber, T.L., and Ausubel, F.M. (1996). Isolation of Arabidopsis genes that differentiate between resistance responses mediated by the *RPS2* and *RPM1* disease resistance genes. Plant Cell **8,** 241–249.

Rich, J.J., Kinscherf, T.G., Kitten, T., and Willis, D.K. (1994). Genetic evidence that the *gacA* gene encodes the cognate response regulator for the *lemA* sensor in *Pseudomonas syringae*. J. Bacteriol. **176,** 7468–7475.

Ritter, C., and Dangl, J.L. (1995). The *avrRpm1* gene of *Pseudomonas syringae* pv. *maculicola* is required for virulence on Arabidopsis. Mol. Plant-Microbe Interact. **8,** 444–453.

Ritter, C., and Dangl, J.L. (1996). Interference between two specific pathogen recognition events mediated by distinct plant disease resistance genes. Plant Cell **8,** 251–257.

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.

Rowley, K.B., Clements, D.E., Mandel, M., Humphreys, T., and Patil, S.S. (1993). Multiple copies of a DNA sequence from *Pseudomonas syringae* pathovar *phaseolicola* abolish thermoregulation of phaseolotoxin production. Mol. Microbiol. **8,** 625–635.

Ryals, J.A., Neuenschwander, U.H., Willits, M.G., Molina, A., Steiner, H.-Y., and Hunt, M.D. (1996). Systemic acquired resistance. Plant Cell **8,** 1809–1819.

Salmond, G.P.C. (1994). Secretion of extracellular virulence factors by plant pathogenic bacteria. Annu. Rev. Phytopathol. **32,** 181–200.

Salmond, G.P.C., and Reeves, P.J. (1993). Membrane traffic wardens and protein secretion in Gram-negative bacteria. Trends Biochem. Sci. **18,** 7–12.

Salmond, G.P.C., Bycroft, B.W., Stewart, G.S.A.B., and Williams, P. (1995). The bacterial "enigma": Cracking the code of cell–cell communication. Mol. Microbiol. **16,** 615–624.

Schulte, R., and Bonas, U. (1992). A *Xanthomonas* pathogenicity locus is induced by sucrose and sulfur-containing amino acids. Plant Cell **4,** 79–86.

Shen, H., and Keen, N.T. (1993). Characterization of the promoter of avirulence gene D from *Pseudomonas syringae* pv. *tomato.* J. Bacteriol. **175,** 5916–5924.

Sheng, J., and Citovsky, V. (1996). Agrobacterium–plant cell DNA transport: Have virulence proteins, will travel. Plant Cell **8,** 1699–1710.

Sory, M.-P., and Cornelis, G.R. (1994). Translocation of a hybrid YopE-adenylate cyclase from *Yersinia enterocolitica* into HeLa cells. Mol. Microbiol. **14,** 583–594.

Staskawicz, B.J., Dahlbeck, D., and Keen, N.T. (1984). Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race specific incompatibility on *Glycine max* (L.) Merr. Proc. Natl. Acad. Sci. USA **81,** 6024–6028.

Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G., and Jones, J.D.G. (1995). Molecular genetics of plant disease resistance. Science **268,** 661–667.

Steinberger, E.M., and Beer, S.V. (1988). Creation and complementation of pathogenicity mutants of *Erwinia amylovora.* Mol. Plant-Microbe Interact. **1,** 135–144.

Swords, K.M.M., Dahlbeck, D., Kearney, B., Roy, M., and Staskawicz, B.J. (1996). Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria avrBs2.* J. Bacteriol., in press.

Takemoto, J.Y. (1992). Bacterial phytotoxin syringomycin and its interaction with host membranes. In Molecular Signals in Plant–Microbe Communications, D.P.S. Verma, ed (Boca Raton, FL: CRC Press), pp. 247–260.

Turner, J.G., and Novacky, A. (1974). The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. Phytopathology **64,** 885–890.

Ullrich, M., and Bender, C.L. (1994). The biosynthetic gene cluster for coronamic acid, an ethylcyclopropyl amino acid, contains genes homologous to amino acid–activating enzymes and thioesterases. J. Bacteriol. **176,** 7574–7586.

Ullrich, M., Penaloza-Vazquez, A., Bailey, A.-M., and Bender, C.L. (1995). A modified two-component regulatory system is involved in temperature-dependent biosynthesis of the *Pseudomonas syringae* phytotoxin coronatine. J. Bacteriol. **177,** 6160–6169.

Van Gijsegem, F., Genin, S., and Boucher, C. (1993). Evolutionary conservation of pathogenicity determinants among plant and animal pathogenic 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 a type III secretion system, encodes eight proteins related to components of the bacterial flagellar biogenesis complex. Mol. Microbiol. **15,** 1095–1114.

Walton, J.D. (1996). Host-selective toxins: Agents of compatibility. Plant Cell **8,** 1723–1733.

Wei, Z.-M., and Beer, S.V. (1995). *hrpL* activates *Erwinia amylovora hrp* gene transcription and is a member of the ECF subfamily of factors. J. Bacteriol. **177,** 6201–6210.

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.

Weiler, E.W., Kutchan, T.M., Gorba, T., Brodschelm, W., Niesel, U., and Bublitz, G. (1994). The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signaling molecules in higher plants. FEBS Lett. **345,** 9–13.

Wengelnkik, K., and Bonas, U. (1996). HrpXv, an AraC-type regulator, activates expression of five of the six loci in the *hrp* cluster of *Xanthomonas campestris* pv. *vesicatoria.* J. Bacteriol. **178,** 3462–3469.

Whalen, M.C., Stall, R.E., and Staskawicz, B.J. (1988). Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA **85,** 6743–6747.

Willis, D.K., Barta, T.M., and Kinscherf, T.G. (1991). Genetics of toxin production and resistance in phytopathogenic bacteria. Experientia **47,** 765–771.

Xiao, Y., and Hutcheson, S. (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. Author's correction. **176,** 6158.

Xiao, Y., Lu, Y., Heu, S., and Hutcheson, S.W. (1992). Organization and environmental regulation of the *Pseudomonas syringae* pv. *syringae* 61 *hrp* cluster. J. Bacteriol. **174,** 1734–1741.

Xiao, Y., Hue, S., Yi, J., 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 *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. J. Bacteriol. **176,** 1025–1036.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T., and Arakawa, M. (1992). Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia*

cepacia (Palleroni and Holmes 1981) comb. nov. Microbiol. Immunol. **36,** 1251–1275.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H., and Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol. Immunol. **39,** 897–904.

Yang, Y., and Gabriel, D.W. (1995). *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. Mol. Plant-Microbe Interact. **8,** 627–631.

Yang, Y., Yuan, Q., and Gabriel, D.W. (1996). Watersoaking function(s) of XcmH1005 are redundantly encoded by members of the *Xanthomonas avr/pth* gene family. Mol. Plant-Microbe Interact. **9,** 105–113.

Yang, Z., Cramer, C.L., and Lacy, G.H. (1992). *Erwinia carotovora* subsp. *carotovora* pectic enzymes: In planta gene activation and roles in soft-rot pathogenesis. Mol. Plant-Microbe Interact. **5,** 104–112.

Young, J.M. (1974). Development of bacterial populations in vivo in relation to plant pathogenicity. N.Z. J. Agric. Res. **17,** 105–113.

Young, S.A., White, F.F., Hopkins, C.M., and Leach, J.E. (1994). AVRXa10 protein is in the cytoplasm of *Xanthomonas oryzae* pv. *oryzae.* Mol. Plant-Microbe Interact. **7,** 799–804.

Zhang, J.-H., Quigley, N.B., and Gross, D.C. (1995). Analysis of the *syrB* and *syrC* genes of *Pseudomonas syringae* pv. *syringae* indicates that syringomycin is synthesized by a thiotemplate mechanism. J. Bacteriol. **177,** 4009–4020.

Zupan, J.R., and Zambryski, P. (1995). Transfer of T-DNA from *Agrobacterium* to the plant cell. Plant Physiol. **107,** 1041–1047.