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PATHOGEN PROFILE

# *Pseudomonas syringae* pv. *tomato*: the right pathogen, of the right plant, at the right time

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## SUMMARY

*Pseudomonas syringae* pv. *tomato* and the closely related pathovar *P. s.* pv. *maculicola* have been the focus of intensive research in recent years, not only because of the diseases they cause on tomato and crucifers, but because strains such as *P. s.* pv. *tomato* DC3000 and *P. s.* pv. *maculicola* ES4326 are pathogens of the model plant *Arabidopsis thaliana*. Consequently, both *P. s.* pv. *tomato* and *P. s.* pv. *maculicola* have been widely used to study the molecular mechanisms of host responses to infection. Analyses of the molecular basis of pathogenesis in *P. s.* pv. *tomato* reveal a complex and intimate interaction between bacteria and plant cells that depends on the coordinated expression of multiple pathogenicity and virulence factors. These include toxins, extracellular proteins and polysaccharides, and the translocation of proteins into plant cells by the type III (Hrp) secretion system. The contribution of individual virulence factors to parasitism and disease development varies significantly between strains. Application of functional genomics and cell biology to both pathogen and host within the *P. s.* pv. *tomato*/*A. thaliana* pathosystem provides a unique opportunity to unravel the molecular interactions underlying plant pathogenesis.

**Taxonomic relationship:** Bacteria; Proteobacteria; gamma subdivision; *Pseudomonadaceae/Moraxellaceae* group; *Pseudomonadaceae* family; *Pseudomonas* genus; *Pseudomonas syringae* species; *tomato* pathovar.

**Microbiological properties:** Gram-negative, aerobic, motile, rod-shaped, polar flagella, oxidase negative, arginine dihydrolase negative, DNA 58–60 mol% GC, elicits the hypersensitive response on tobacco.

**Host range:** Primarily studied as the causal agent of bacterial speck of tomato and as a model pathogen of *A. thaliana*, although it has been isolated from a wide range of crop and weed species.

**Disease symptoms:** Tomato (*Lycopersicon esculentum*): Brown-black leaf spots sometimes surrounded by chlorotic margin; dark superficial specks on green fruit; specks on ripe fruit may become sunken, and are surrounded by a zone of delayed ripening. Stunting and yield loss, particularly if young plants are infected. Reduced market value of speckled fruit. *A. thaliana*: Water-soaked, spreading lesions, sometimes surrounded by chlorotic margin.

**Epidemiology:** Seed borne. Survives as a saprophyte in plant debris, soil and on leaf surfaces. Dispersed by aerosols and rain splash. Development of disease symptoms favoured by leaf wetness and cool temperatures (55–77 °F/13–25 °C).

**Disease control:** Pathogen-free seed and transplants. Resistant and tolerant cultivars. Sanitation, rotation, and drip irrigation to reduce leaf wetness. Some measure of control with bactericides (copper, streptomycin).

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## INTRODUCTION

*Pseudomonas syringae* pv. *tomato* is the causal agent of bacterial speck of tomato. Like many *syringae* pathovars it is found growing epiphytically on a wide range of plants, although field populations decline in the absence of a susceptible host. Serious disease outbreaks are relatively infrequent, and are favoured by high leaf wetness, cool temperatures and cultural practices that allow bacteria to be disseminated between host plants. There are three main reasons for the high level of scientific interest in this pathogen. Firstly, as an easily cultured Gram-negative bacterial pathogen it is amenable to a wide range of molecular genetic and cell biology techniques, facilitating the experimental identification and manipulation of putative pathogenicity and virulence factors. Second, tomato (*Lycopersicon esculentum*) is similarly amenable to transformation and genetic analysis, facilitating the isolation and characterization of plant genes involved in host responses. But third, and perhaps most significantly, many strains of *P. s.* pv. *tomato* and the closely related pathogen *P. s.* pv. *maculicola* are pathogenic on the model plant

*Arabidopsis thaliana*, and certain strains exhibit race-cultivar specificity on this host, thus providing a model pathosystem for studying both compatible and incompatible host–pathogen interactions.

Both *A. thaliana* and *P. s.* pv. *tomato* are currently the subjects of comprehensive genomic and functional genomic analyses that could greatly increase our understanding of bacterial pathogenesis and plant responses to bacterial infection. It is time to review the biology of *P. s.* pv. *tomato* and to consider how current advances in genomics, proteomics and cell biology can be most profitably applied to advance our understanding of this model pathogen.

## *P. S.* PV. *TOMATO* AND *P. S.* PV. *MACULICOLA* ARE MEMBERS OF *P. SYRINAGE* GENOSPECIES III AND CAUSE DISEASES OF TOMATOES AND CRUCIFERS

*P. s.* pvs. *tomato* and *maculicola* have been shown by DNA pairing analyses to belong to the same genomic species—*P. syringae* genospecies III. This conclusion is supported by RFLP, RAPD and AFLP techniques (Clerc *et al*., 1998; Manceau and Horvais, 1997). Other pathovars attributed to genomospecies III include pvs. *persicae*, *viburni*, *berberidis*, *apii*, *delphinii*, *passiflorae*, *philadelphi*, *ribicola* and *primulae* (Gardan *et al*., 1999; Manceau and Horvais, 1997). Almost all strains of *P. s.* pv. *maculicola* tested have been shown to be pathogenic on both crucifers and tomato, while many *P. s.* pv. *tomato* strains are pathogenic only on tomato. *P. s.* pv. *tomato* DC3000, one of the most widely studied strains of *P. s.* pv. *tomato,* is pathogenic towards both tomato and crucifers (Cuppels and Ainsworth, 1995). Several strains of both pathovars have been shown to be pathogenic to varying degrees towards *A. thaliana* (Davis *et al*., 1991).

The necrotic and chlorotic symptoms produced by *P. s.* pv. *tomato* on host plants are quite distinctive (Fig. 1A,B), as summarized at the beginning of this article, although they are occasionally confused with the symptoms of bacterial leaf spot caused by *Xanthomonas campestris* pv. *vesicatoria*. Bacteria enter the intercellular spaces of leaves through natural openings such as stomata and multiply endophytically and asymptomatically prior to symptom development (Fig. 1C,D). Bacteria also infect green tomato fruit, causing necrotic specks and delayed ripening, but the majority of molecular genetic analyses of *P. s.* pv. *tomato* pathogenesis have used bacterial multiplication in leaf tissue as a measure of virulence and parasitic competence.

## THE HRP (TYPE III) PROTEIN SECRETION PATHWAY

The ability of *P. s.* pv. *tomato* to multiply endophytically in susceptible hosts and to elicit the rapid localized defence response known as the hypersensitive response (HR) in nonhost and resistant plants, is dependent on a type III protein secretion pathway encoded by a cluster of conserved *hrp/hrc* genes. Current evidence indicates that the type III pathway of *P. s.* pv. *tomato* provides a mechanism by which proteins can be secreted into the apoplast to interact with the cell wall and plasma membrane and translocated directly into the cytoplasm of host cells (Galan and Collmer, 1999). Type III pathways have a central role in the pathogenesis of many bacterial pathogens of plants and animals and have been intensively characterized at a molecular level. For the purposes of this article I describe only those features relevant to an understanding of the biology of *P. s.* pv. *tomato*, and refer the reader to one of many excellent reviews on this topic (Cornelis and van Gijsegem, 2000; Galan and Collmer, 1999; Hueck, 1998).

### (i) The type III secretion pathway

Structurally, the type III pathway combines an inner membrane complex resembling the inner membrane complex of the flagellar protein secretion pathway, with an outer membrane component resembling proteins involved in type II protein secretion and type IV pilus biogenesis. The genes encoding the core components of the type III secretion pathway are highly conserved across a wide range of bacteria and are known as *hrc* genes. Plant pathogens carrying type III secretion pathways can be subdivided into group I (*P. syringae* and *Erwinia* spp.) and group II (*R. solanacearum* and *Xanthomonas* spp.) (Alfano and Collmer, 1997). *P. s.* pv. *tomato* possesses a group I type III pathway, which is more closely related to that of *Yersinia* spp. than *Xanthomonas* spp. Type III secretion in *P. s.* pv. *tomato* is also dependent on *hrp* genes, which encode a mixture of structural and regulatory proteins associated with type III secretion that are not conserved across a wide range of bacteria, although many of the *hrp* genes present in *P. s.* pv. *tomato* are conserved in other group I plant pathogens.

Protein secretion by type III pathways is fairly promiscuous, and it has been demonstrated that *Yersinia, Pseudomonas* and *Erwinia* spp. can secrete heterologous proteins from different strains and species (Anderson *et al*., 1999; Ham *et al*., 1998). Targeting of at least some proteins to the type III secretion pathway appears to be encoded at an RNA level, and probably involves a stem-loop structure traversing both the Shine–Dalgarno sequence and the translation start AUG codon. mRNA-dependent targeting has been experimentally demonstrated for the type III-secreted AvrPto protein of *P. s.* pv. *tomato* (Anderson *et al*., 1999). Type III secretion in *P. s.* pv. *tomato* is subject to environmental regulation at both a transcriptional and post-transcriptional level. *hrp* gene expression and type III secretion of proteins such as HrpZ and HrpW can be induced in apoplast-mimicking medium and *in planta*, but particular levels of pH and temperature are needed in order to stimulate secretion of AvrPto in culture (van Dijk *et al*., 1999).

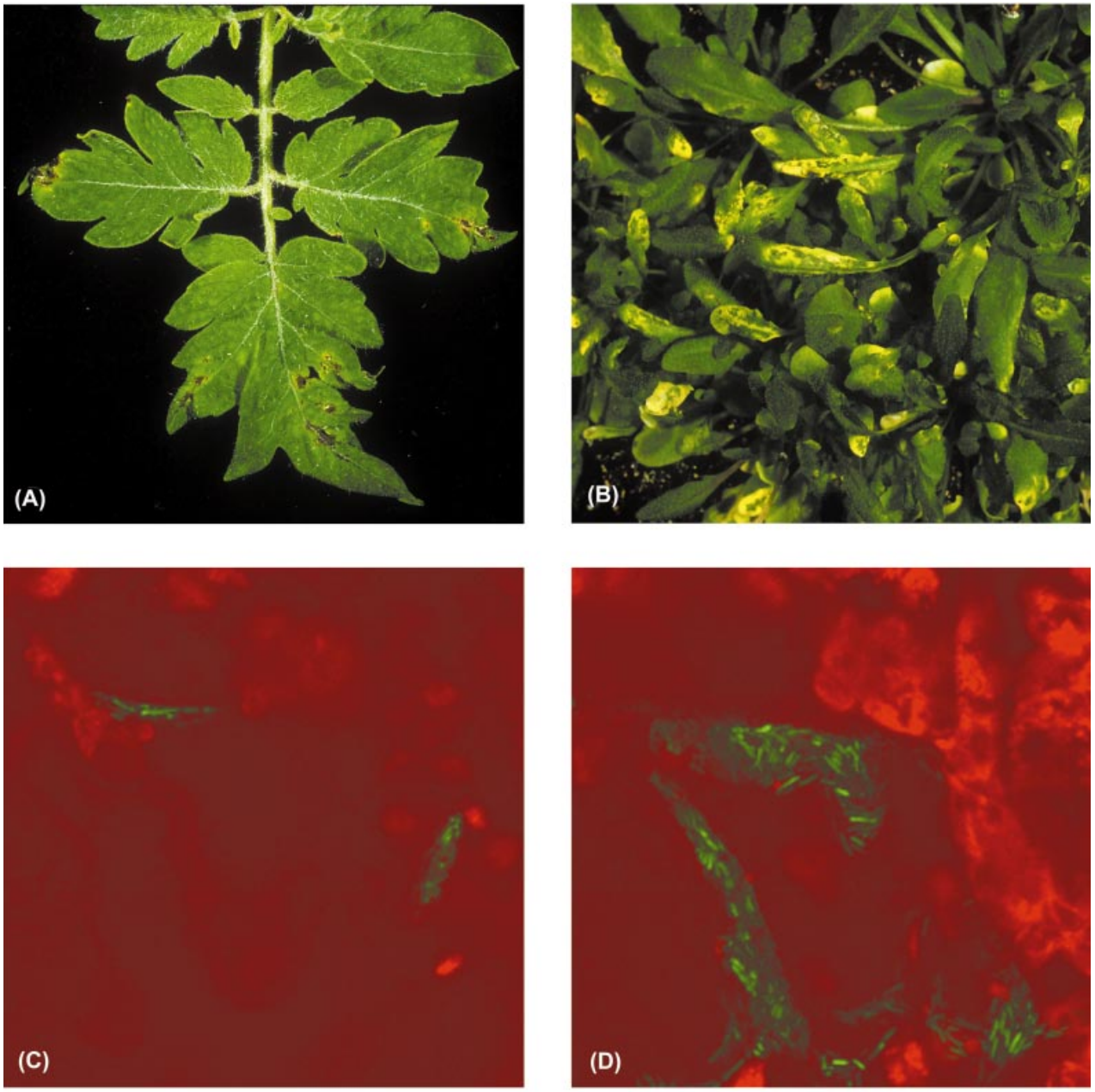
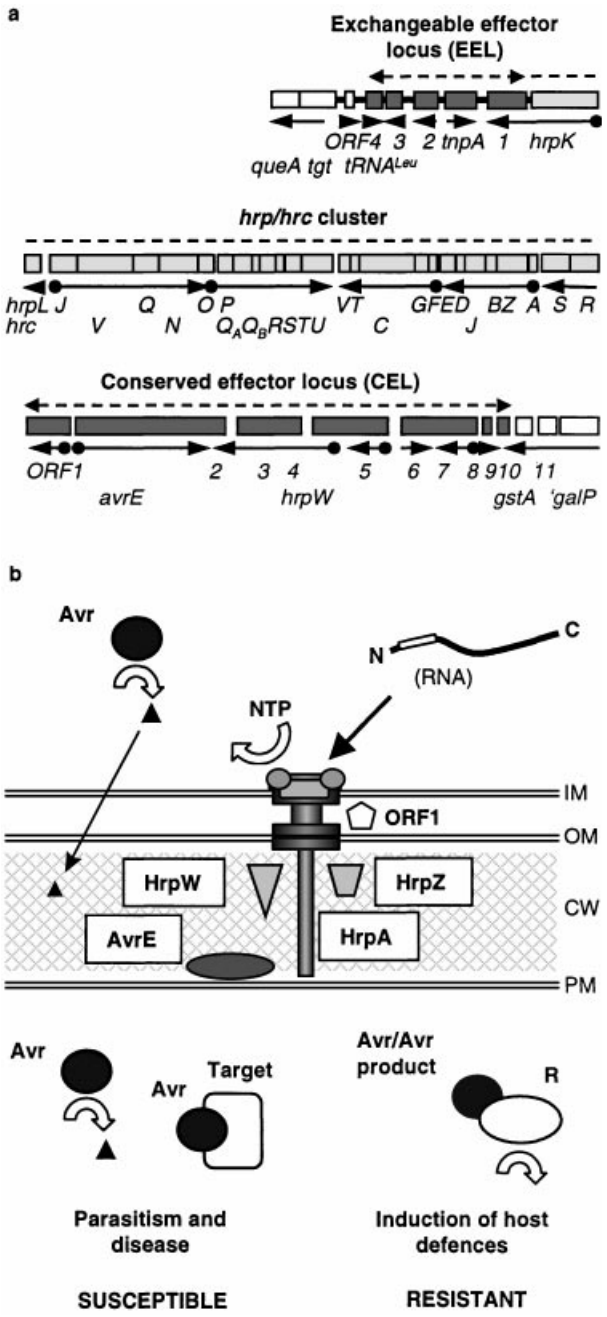



**Fig. 1** (A) and (B) Symptoms caused by *P. s.* pv. *tomato* DC3000 on tomato (*L. esculentum* cv. Moneymaker) (A) and *A. thaliana* ecotype Columbia (B) 7 days after spray inoculation. (C) and (D) Optical sections obtained using confocal microscopy of a tomato leaf 4 days after infection with *P. s.* pv. *tomato* carrying a plasmid with *hrpA* promoter fused to stable GFP. Bacteria in and below the stomata are strongly induced. (C) *P. s.* pv. *tomato* colonizing the stomatal openings of a tomato leaf. (D) *P. s.* pv. *tomato* colonizing the intercellular space below the stomate. Images (C) and (D) provided by M. Romantschuk, T. Boureau and S. Taira.

### (ii) The Hrp pathogenicity island

The type III secretion genes of many bacterial pathogens are frequently clustered with other virulence and pathogenicity factors in pathogenicity islands (PAIs) or on large extrachromosomal elements. The organization of the flanking regions of the *P. s.* pv. *tomato* DC3000 Hrp pathogenicity island has been described in a recent paper by Alfano *et al.* (2000) (reviewed in Collmer *et al.*, 2000). The Hrp PAI has three distinct components (Fig. 2A).

- A central cluster of *hrp/hrc* genes that is highly conserved in *P. syringae* and in other group I bacteria. Essential for HR and growth *in planta*.




• A conserved effector locus (CEL), which contains at least seven ORFs that are conserved between *P. s.* pv. *tomato* DC3000 and *P. s.* pv. *syringae* B728a, including type III secreted proteins such as HrpW and AvrE that are also conserved in other group I bacteria. Deletion results in a delayed nonhost HR (but does not affect the race-specific HR) and strongly reduced growth *in planta*.

• An exchangeable effector locus (EEL), which starts 3 nt downstream of *hrpK* and encodes several ORFs that are not conserved in other pathovars of *P. syringae*, at least one of which is Hrp secreted. Not required for HR, but deletion reduces growth *in planta*.

The GC content of the ORFs in the EEL is unusually low compared to the average *P. syringae* GC content (59–61%) and the EEL is bordered by a $tRNA^{LEU}$ gene. The high variability of the EELs in different pathovars, and even different strains of the same pathovar, suggests that it is subject to a high degree of recombination and variation. An interesting line of investigation for the future will be to characterize the degree of diversity and the relative contribution to growth and pathogenicity of the EELs and CELs in a range of different *P. s.* pv. *tomato* and *maculicola* strains.

### (iii) Type III secretion and translocation are dependent on a Hrp-dependent pilus

Type III secretion in *P. s.* pv. *tomato* is associated with the production of a *hrp*-dependent pilus, 6–8 nm in diameter, that is required both for secretion in culture and for the translocation of proteins into plant cells (Roine *et al.*, 1997; Wei *et al.*, 2000). It has been speculated that one function of the HrpA pilus may be to direct protein translocation across the cell wall. The structural protein of the *hrp* pilus is HrpA, a small *hrp* cluster-encoded protein that displays a high degree of divergence between pathovars, which may reflect its extracellular location and selection pressure to evade host surveillance mechanisms (Preston *et al.*, 1995; Roine *et al.*, 1997). Purified HrpA can reassemble into pili *in vitro* (Roine *et al.*, 1998a). Intriguingly, mutational analysis of *hrpA* has shown that the amino

**Fig. 2** (A) The Hrp pathogenicity island (PAI) of *P. s.* pv. *tomato* DC3000. Schematic diagram of tripartite PAI, reading continuously from left to right, top to bottom. Arrows indicate direction of transcription with solid circles indicating location of putative HrpL regulated promoters (based on Alfano *et al.* (2000) and Collmer *et al.* (2000) ). (B) Model of Hrp secretion in *P. s.* pv. *tomato* showing putative destinations and biological activities of Avr proteins (black circles) and type III accessory proteins (as labelled). At least some type III-secreted proteins appear to be targeted for secretion in a mRNA-dependent manner (top right). The biological function of most Avr proteins remains unclear, although AvrD has been shown to direct the production of syringolide elicitors (top left, Avr-synthesized molecules represented by black triangles). At least some Avr proteins appear to be translocated directly into host cells (bottom). Translocated Avr proteins may direct the synthesis of low molecular weight molecules using host resources, or they may interact directly with host proteins, nucleic acids or membranes to promote parasitism (bottom left). In an incompatible interaction Avr proteins or Avr-derived molecules are recognized by host surveillance mechanisms. This recognition event may involve a direct interaction between *avr* and *R* gene products (R) that initiates a signal transduction cascade leading to induction of local and systemic host defences (bottom right). (Abbreviations: IM, bacterial inner membrane; OM, bacterial outer membrane; CW, plant cell wall; PM, plant plasma membrane).

**Table 1** Avr proteins and type III secreted/accessory proteins identified in *P. s.* pvs. *tomato* and *maculicola*.

| Name | Origin | Location | Description | Cellular location, phenotype and function |
|---|---|---|---|---|
| HrpZ[1] | *P. s.* pv. *tomato* DC3000* | Chromosome—*hrp/hrc* gene cluster | 370 amino acids (36.5 kDa) Glycine-rich 'harpin' | Apoplast/cell wall, putative type III translocation accessory protein.[2] Purified protein elicits HR in tobacco. Not required for HR or pathogenicity |
| HrpW[2] | *P. s.* pv. *tomato* DC3000* | Chromosome—conserved effector locus (CEL) | 425 amino acids (42.9 kDa) Harpin-like N-terminal, pectate lyase-like C-terminal | Apoplast/cell wall, putative type III translocation accessory protein. Binds pectate. Purified protein elicits HR in tobacco. Not required for HR or pathogenicity |
| AvrA[3,4] | *P. s.* pv. *tomato* PT23 | Chromosome? | 907 amino acids (100 kDa). AvrA allele in *P. s.* pv. *glycinea* | Host cytoplasm? Confers avirulence on soybean. Mutant reduced in virulence |
| AvrD[3,4,5] | *P. s.* pv. *tomato* PT23 | Plasmid pPT23B | 311 amino acids (34 kDa). AvrD alleles in *P. s.* pv. *glycinea* and other *syringae* pvs. | Synthesis of syringolide elictors[6,7]. Rpg4 (*R*)—soybean[8,9]. Confers avirulence on soybean. No effect on virulence[4,10] |
| AvrE[4,11,12] | *P. s.* pv. *tomato* DC3000 & PT23* | Chromosome—CEL | 1795 amino acids (195 kDa) Homologous to DspE/DspA from *Erwinia* spp. Secreted in culture | Apoplast—plasma membrane? Confers avirulence on soybean. PT23 mutant reduced in virulence, DC3000 mutant no effect on virulence. No effect on HR |
| AvrPto[13,14] | *P. s.* pv. *tomato* DC3000 & JL 1065 | Chromosome | 164 amino acids (18.3 kDa) Acylation motif | Host cytoplasm—membrane. *Pto* (*R*)—tomato[15]. Interacts with Api proteins[16] Confers avirulence on soybean and tomato cultivars. Enhances virulence and necrosis on susceptible host[17,18] |
| AvrRpt2[19] | *P. s.* pv. *tomato* JL 1065 | Probably chromosome | 256 amino acids (28.2 kDa) Hydrophilic protein | Host cytoplasm. *RPS2* (*R*)—*A. thaliana*[20,21]. Confers avirulence on *A. thaliana*, bean and soybean. Transgenic expression causes browning of *A. thaliana* |
| AvrRpm1/ AvrPmaA1[24,25] | *P. s.* pv. *maculicola* M2 | Plasmid (chromosomal alleles in some strains) | 220 amino acids (24 kDa) Acylation motif. AvrPpiA1 allele in *P. s.* pv. *pisi* | Host cytoplasm—plasma membrane[25]. *RPM1* (*R*)—*A. thaliana*[26]. Confers avirulence on *A. thaliana*, soybean, pea (*R2*), bean (*RN1/RN2*). Required for full virulence on *A. thaliana* |
| EEL ORF1[12] | *P. s.* pv. *tomato* DC3000 | Chromosome—exchangeable effector locus (EEL) | 466 amino acids (50 kDa) No similarity to known proteins | Hrp-secreted protein. Function unknown |
| CEL ORF1[12] | *P. s.* pv. *tomato** DC3000 | Chromosome—CEL | 495 amino acids (54 kDa) Similar to *E. coli* MltD. Lysozyme-like domain | Type III accessory protein? May facilitate insertion of type III system through peptidoglycan layer. Mutants have no obvious phenotype |

*Probably common to many, if not all, strains of *P. syringae*. (*R*) Corresponding resistance gene, where known. References: [1]Preston *et al.*, 1995; [2]Charkowski *et al.*, 1998; [3]Kobayashi *et al.* (1989); [4]Lorang *et al.*, 1994; [5]Kobayashi *et al.* (1990); [6]Keen *et al.* (1990); [7]Midland *et al.*, 1993; [8]Keen and Buzzell (1991); [9]Ji *et al.*, 1998; [10]Murillo *et al.*, 1994; [11]Lorang and Keen, 1995; [12]Alfano *et al.*, 2000; [13]Ronald *et al.* (1992); [14]Salmeron and Staskawicz (1993); [15]Martin *et al.*, 1993; [16]Bogdanove & Martin, 2000; [17]Chang *et al.*, 2000; [18]Tobias *et al.*, 1999; [19]Innes *et al.* (1993); [20]Bent *et al.*, 1994; [21]Mindrinos *et al.*, 1994; [22]McNellis *et al.* (1998); [23]Whalen *et al.* (1991); [24]Ritter and Dangl (1995); [25]Nimchuk *et al.*, 2000; [26]Grant *et al.*, 1995.

terminal half of HrpA is not required for pilus assembly and interaction with the plant, although insertions in the 5′ upstream region and first codons abolish protein production and decrease mRNA stability (Taira *et al.*, 1999). HrpA is required for the full expression of type III genes at the level of the Hrp response regulator genes *hrpRS*, since ectopic expression of *hrpRS* can restore normal gene expression to a HrpA mutant (Wei *et al.*, 2000).

### (iv) Type III-secreted harpins—accessory proteins for type III secretion?

The Hrp PAI of *P. s.* pv. *tomato* encodes two proteins, HrpZ and HrpW, that belong to the family of glycine-rich, type III secreted proteins known as harpins (Fig. 2, Table 1) (Charkowski *et al.*, 1998; Preston *et al.*, 1995). HrpW encodes a protein with an N-terminal harpin-like domain and a C-terminal pectate lyase-like

domain that has pectate-binding activity (Charkowski *et al.*, 1998). HrpZ does not have a recognizable pectate-binding domain, but HrpZ from *P. s.* pv. *syringae* has been shown to interact with plant cell walls (Hoyos *et al.*, 1996). Both HrpZ and HrpW elicit a HR-like response when purified proteins are infiltrated into plants, and truncated fragments of HrpZ from *P. s.* pv. *syringae* and the N-terminal half of HrpW retain elicitor activity (Alfano *et al.*, 1996; Charkowski *et al.*, 1998). However, the biological significance of the harpin-induced HR remains unclear, as bacteria secreting only HrpZ fail to elicit the HR (Alfano *et al.*, 1996). Neither HrpZ nor HrpW mutants are strongly impaired in pathogenesis and HR elicitation, but a HrpZ/HrpW double mutant is significantly reduced in its ability to elicit the HR (Charkowski *et al.*, 1998). It has been suggested that harpins function collectively as type III accessory proteins, facilitating the translocation of type III secreted proteins across the cell wall, perhaps by assisting or interacting with the HrpA encoded *hrp* pilus (Fig. 2B). The weak phenotype of individual harpin mutants may reflect the presence of multiple harpins with redundant activity, but it remains to be shown whether *P. s.* pv. *tomato* harpin-like proteins are collectively required for HR elicitation.

## TYPE III-SECRETED PROTEINS AND HOST SPECIFICITY

Ultimately, the biological function of type III secretion is dependent on type III secreted proteins. As type III secretion pathways have been most frequently characterized from pathogenic bacteria, it has become customary to regard type III secreted proteins as virulence factors, type III accessory proteins or both. However, the discovery of functional type III pathways in nonpathogenic and symbiotic bacteria such as *P. fluorescens* and *Rhizobium* spp. challenges this narrow definition of type III function (Freiberg *et al.*, 1997; Meinhardt *et al.*, 1993; Rainey, 1999; G. Preston, N. Bertrand and P. Rainey, unpublished data; reviewed in Preston *et al.*, 1998a). Since homologues of at least some type III secreted proteins are found in both pathogens and symbionts it is more accurate to regard type III-secreted proteins simply as proteins that promote the interaction of a bacterium with a eukaryotic host. Defining the appropriate nomenclature for type III secreted proteins is a continuing challenge and various proposals have been put forward, although none of them have been universally adopted (Alfano *et al.*, 1997; Jackson *et al.*, 1999; Vivian and Mansfield, 1993).

The phenotypes attributed to type III secreted proteins of *P. s.* pv. *tomato* fall into three categories:

- Type III *translocation and accessory* proteins are associated with the type III secretion machinery. Mutants are generically affected in their ability to elicit the HR and may be affected in multiplication and virulence in a susceptible host.
- Type III *effector* proteins are not required for type III secretion and translocation and have a biological role in plant–bacteria interactions. Diverse type III effectors may function collectively to confer parasitism on a susceptible host. Mutant phenotypes can be quite variable and are dependent on genetic background.
- Type III *avirulence* (Avr) proteins elicit the HR when recognized by host plants carrying a corresponding resistance gene (*R* gene), generally resulting in the cessation of bacterial growth within 12–24 h after inoculation. Avr mutants may be virulent on a previously resistant host, dependent on genetic background.

These categories are not mutually exclusive, as many 'Avr' proteins are believed to function as effector proteins on compatible host plants, and it is possible that host surveillance mechanisms may evolve to recognize signals derived from extracellular components of the type III secretion machinery. It is also important to recognize that the source of an Avr signal may not necessarily be type III-dependent. A summary of the major type III secreted and Avr proteins identified from *P. s.* pv. *tomato* to date is given in Table 1. Many groups have used Southern hybridization to demonstrate that alleles of genes encoding type III secreted proteins are conserved in numerous strains not listed in Table 1. Recent reviews provide a comprehensive overview of this topic (Kjemtrup *et al.*, 2000; Vivian and Gibbon, 1997; White *et al.*, 2000), so only highlights of research into selected *P. s.* pv. *tomato* Avr/effector proteins are outlined below.

### (i) AvrD

AvrD remains the only Avr protein for which a biochemical function has been clearly defined. AvrD directs the synthesis of low molecular weight syringolide elicitors which elicit the HR on soybean (Midland *et al.*, 1993). Different alleles of *avrD* direct the synthesis of different host-specific syringolides (Yucel *et al.*, 1994a,b). Plasmid-borne homologues of *avrD* are widespread among pseudomonads and there is at least one chromosomal *avrD* allele in *P. s.* pv. *glycinea* race 1 (Keith *et al.*, 1997; Yucel *et al.*, 1994a). However, an *avrD* mutant of *P. s.* pv. *tomato* PT23 and strains lacking the *avrD* containing plasmid pPT23B are not impaired in virulence on tomato (Lorang *et al.*, 1994; Murillo *et al.*, 1994). Recently, Ji *et al.* (1998) have identified a syringolide-binding protein, Rpg4, from resistant soybean plants. Although AvrD is co-regulated with *hrp* gene expression it is not known whether AvrD is translocated into host cells by the type III secretion machinery or what its biological function is in *P. s.* pv. *tomato* (Shen and Keen, 1993).

### (ii) AvrPto

AvrPto was the first Avr protein for which a corresponding plant resistance gene (*R* gene) was identified—the *Pto* gene of tomato, which encodes a protein kinase (Martin *et al.*, 1993). Although there is a direct interaction between AvrPto and Pto in resistant plants (Scofield *et al.*, 1996; Tang *et al.*, 1996), the cellular target of AvrPto in susceptible plants appears to be

quite distinct. Bogdanove and Martin (2000) have used yeast two and three-hybrid screens to show that there are proteins which interact specifically with Pto when it binds AvrPto (Adi proteins), and that there are four classes of proteins that interact directly with AvrPto in a susceptible host (Api proteins). Api proteins may be host targets for AvrPto, or they may be host proteins that modify AvrPto subsequent to translocation into the host cell. Mutational analysis of AvrPto shows that the avirulence function of AvrPto can be functionally separated from the virulence function (Shan *et al*., 2000). Recent experiments have shown that AvrPto causes necrosis when expressed in transgenic plants, and that it enhances the ability of *P. s.* pv. *tomato* T1 to induce necrosis and multiply *in planta* when inoculated into tomato plants lacking Pto or Prf (Chang *et al*., 2000; Tobias *et al*., 1999).

### (iii) AvrRpt2 and AvrRpm1

The *A. thaliana R* genes *RPS2* and *RPM1* conferring resistance to the AvrRpt2 protein of *P. s.* pv. *tomato and* the AvrRpm1 (AvrPmaA1) protein of *P. s.* pv. *maculicola* have also been cloned (Bent *et al*., 1994; Grant *et al*., 1995; Mindrinos *et al*., 1994). Experiments have shown that there is interference between the two resistance genes and between the two Avr proteins when they are expressed in the same host, with AvrRpt2 being epistatic to AvrRpm1 (Reuber and Ausubel, 1996; Ritter and Dangl, 1996). This result raises important questions with regard to the theory that Avr proteins function collectively to promote pathogenicity, although it is interesting to note that alleles of these two Avr proteins have not been observed to be present in the same strain. A recent study of a large virulence plasmid from *P. s.* pv. *phaseolicola* found that additional HR responses were observed in a plasmid cured strain, indicating that some effector activities may be epistatic to Avr activities (Jackson *et al*., 1999).

AvrRpm1 and certain other Avr proteins with a conserved acylation site are localized to the plasma membrane in a myristoylation-dependent manner (Nimchuk *et al*., 2000). It is not clear whether AvrRpt2 is also localized to the membrane, but it has been shown that AvrRpt2 is N-terminally processed by an intracellular protease following secretion *in planta*, and when expressed in transgenic plants. The N-terminal domain of AvrRpt2 appears to contain the signal for translocation by *P. s.* pv. *tomato*. This demonstration of intracellular processing of a bacterially delivered Avr protein provides some of the most compelling evidence to date for the translocation of Avr proteins into plant cells (Mudgett and Staskawicz, 1999).

## THE ROLE OF CORONATINE IN PATHOGENICITY AND VIRULENCE

Toxins have long been identified as central factors in parasitism and pathogenesis of *P. syringae*. *P. s.* pv. *tomato* and *P. s.* pv. *maculicola* are primarily associated with production of the phytotoxin coronatine (COR) and coronatine derivatives, but a phaseolotoxin-like substance has also been characterized from some *P. s.* pv. *tomato* strains (Bagdache *et al*., 1990), and certain strains of *P. s.* pv. *tomato* have been shown to have antimicrobial activity (Voelksch and Weingart, 1998). *P. s.* pv. *tomato* strains can be characterized as those that produce either toxin, both, or none at all. The genetics and biochemistry of toxin production by *P. syringae* has recently been reviewed in depth by Bender *et al*. (1999).

The primary symptom associated with coronatine production is chlorosis, although in *A. thaliana* exogenous coronatine induces anthocyanin expression at the site of inoculation, giving the tissue a strong purple hue (Bent *et al*., 1992). Coronatine has also been shown to induce hypertrophy, ethylene production and the synthesis of proteinase inhibitors, and to inhibit root elongation (Bender *et al*., 1999). Coronatine mimics some of the activities of the phytohormone methyl jasmonate (MeJA), and *A. thaliana coi1* mutants are insensitive to both coronatine and MeJA (Feys *et al*., 1994). However, unlike MeJA, coronatine induces chlorosis of host tissues, and causes shrinkage of chloroplasts (Palmer and Bender, 1995).

The precise role of coronatine in plant parasitism remains unclear, although there is increasing evidence that coronatine may function in part by suppressing the induction of defence-related genes (Budde and Ullrich, 2000; Mittal and Davis, 1995). Some *P. s.* pv. *tomato* and *maculicola* strains expressing coronatine have been shown to reach and sustain higher populations *in planta*, and for certain strains, such as DC3000, coronatine appears to be vital for pathogenesis (Bender *et al*., 1987; Mittal and Davis, 1995; Penaloza-Vazquez *et al*., 2000; Tamura *et al*., 1998). The virulence of at least some *P. s.* pv. *maculicola* strains is not dependent on coronatine, as four $COR^-$ strains were able to cause water-soaked or necrotic lesions without chlorosis (Wiebe and Campbell, 1993).

The coronatine biosynthetic clusters of many *P. s.* pv. *tomato* strains and at least one pv. *maculicola* strain are plasmid-borne, as are coronatine clusters in strains of pvs. *atropurpurea*, *glycinea* and *morsprunorum* (Bender *et al*., 1999), while the coronatine gene clusters of *P. s.* pv. *tomato* DC3000 and several other strains in pvs. *tomato* and *maculicola* are chromosomal (Cuppels and Ainsworth, 1995; Moore *et al*., 1989). Although the coronatine cluster is highly conserved, there are distinct differences between plasmid-borne and chromosomal clusters. It has been speculated that the distribution of toxin genes between chromosome and plasmids may reflect differences in levels of toxin synthesis, or the ecological role of the toxin genes, as plasmid-borne genes may be more easily gained and lost (Cuppels and Ainsworth, 1995). In this respect it is interesting to note that $COR^-$ mutants of the plasmid-borne coronatine genes in *P. s.* pv. *tomato* PT23.2 retain their ability to cause necrotic lesions on tomato and

multiply *in planta*, while COR⁻ mutations of the chromosomal coronatine genes in *P. s.* pv. *tomato* DC3000 abolish symptom production following the spray or dip inoculation of tomato leaves and greatly reduce multiplication *in planta* (Mittal and Davis, 1995; Penaloza-Vazquez *et al.*, 2000).

## PLASMIDS, RECOMBINATION AND GENOMIC PLASTICITY

The variability of the effector loci flanking the Hrp PAI, the presence of Avr proteins and toxin biosynthesis genes on plasmids, and the multiple alleles and diverse phenotypes of type III secreted effectors and accessory proteins give some indication of the degree of genomic flexibility found in *P. s.* pv. *tomato*. Direct evidence of the dynamic nature of the *P. s.* pv. *tomato* genome comes from studies of plasmids, insertion elements and bacteriophage sequences in *P. s.* pv. *tomato*.

### (i) Four plasmids encode toxin production, copper resistance and host specificity in *P. s.* pv. *tomato* PT23

Isolates of *P. s.* pv. *tomato* and *P. s.* pv. *maculicola* can contain from none to seven plasmids ranging from 11 to 105 kb (Wiebe and Campbell, 1993). *P. s.* pv. *tomato* DC3000 contains a 68-kb plasmid, but the best studied strain with regard to plasmid structure and function is *P. s.* pv. *tomato* PT23, which contains four plasmids of varying size. pPT23A (95–103 kb) carries the 30 kb coronatine biosynthesis cluster and has been shown to contain homologues of the UV resistance genes *rulAB*. pPT23B (71–83 kb) encodes the *avrD* gene at the beginning of a five ORF operon. pPT23C (59–67 kb) and pPT23D (37–39 kb) have both been linked to copper resistance (Bender *et al.*, 1999; Keun and Cooksey, 1993; Murillo *et al.*, 1994). The plasmids in *P. s.* pv. *tomato* share a large amount of repeated DNA and up to five different native plasmids have been shown to coexist within the same cell (Murillo and Keen, 1994). Conjugation, duplication and recombination of plasmid DNA probably occurs at a high frequency in *P. s.* pv. *tomato*. There is evidence that modifications of *repA* and low conservation of maintenance determinants facilitate the suppression of plasmid incompatibility in the pPT23A family found in *P. s.* pv. *tomato* and other pathovars, allowing plasmids to coexist and enhancing genomic plasticity (Sesma *et al.*, 1998).

### (ii) IS elements and bacteriophage sequences

There is mounting evidence for the importance of transposable elements and bacteriophage sequences in generating phenotypic diversity in *P. s.* pv. *tomato*. In addition to the putative recombination sites identified in the Hrp PAI, Kim *et al.* (1998) have discovered that many *P. s.* pv. *tomato* avirulence genes are flanked by such sequences, including the IS element IS*1240* at the 5′ region of *P. s.* pv. *tomato avrD* (Hanekamp *et al.*, 1997), and a Tn*501* sequence flanking *avrRpm1* and its homologue from *P. s.* pv. *pisi avrPpiA1*. *avrPto* is adjacent to a bacteriophage protein, but as yet there is no indication of IS or phage sequences flanking *avrRpt2*.

Perhaps most intriguingly, in a recent review Kjemtrup *et al.* (2000) describes an unpublished study in which excision of *avrRpm1* from the chromosome of *P. s.* pv. *maculicola* M6 was observed when bacteria were introduced into resistant plants. The excised *avrRpm1* gene was present on a plasmid, but insertion of Tn3 into *avrRpm1* in 5% of plasmid-borne copies, restored virulence (P. Marchesini, S. Kjemtrup, L. Rohmer and J. Dangl, unpublished data).

## ADDITIONAL VIRULENCE AND PATHOGENICITY FACTORS

Type III secretion and toxin biosynthesis have been the primary pathogenicity and virulence factors characterized at a molecular level in *P. s.* pv. *tomato* to date, but a limited amount of research has identified other potential virulence and colonization factors, including ice nucleation, protease synthesis, utilization of citric and malic acid, and many factors linked to epiphytic survival. Epiphytic growth is a key part of the *P. syringae* life-cycle, and successful epiphytes have been shown to possess a range of traits including chemotaxis, attachment, microcolony formation, nutrient acquisition, antibiosis and resistance to UV stress (Beattie and Lindow, 1995; Beattie and Lindow, 1999; Hirano and Upper, 1990). The type IV pili of *P. s.* pv. *tomato*, encoded by the *pilA* gene, have been linked to both epiphytic growth and UV tolerance (Roine *et al.*, 1998b).

Extracellular polysaccharides (EPS) play an important role in the pathogenesis of many bacteria both in terms of direct interactions with host cells and in conferring resistance to oxidative stress. Alginate has been characterized as an important virulence factor for both *P. aeruginosa* and *P. s.* pv. *syringae* 3525 (Jing *et al.*, 1999). Although *P. s.* pv. *tomato* has been reported to produce alginate (Gross and Rudolph, 1987) the potential contribution of alginate to pathogenicity and virulence in *P. s.* pv. *tomato* has not been investigated.

## REGULATION AND COORDINATION OF PATHOGENICITY AND VIRULENCE FACTORS

The regulatory cascades that directly induce *hrp* and coronatine expression are well-defined and conserved in different *P. syringae* pathovars (Bender *et al.*, 1999; Hutcheson *et al.*, 1996), but the molecular connection between environmental signals and the positive regulation of gene expression is not well understood.

There is little experimental evidence for the role of global regulators and sigma factors in coordinating gene expression

in *P. s.* pv. *tomato*. However, $\sigma^{54}$ (RpoN) has been shown to be required for *hrp, avr* and coronatine expression in *P. s.* pv. *maculicola* ES4326, as well as for motility and the ability to utilize nitrate, urea dicarboxylic acids and several amino acids as nitrogen sources (Hendrickson *et al*., 2000a,b). Very recently, a study by Penaloza-Vazquez *et al*. (2000) has shown that a polar *hrcC* mutant of *P. s. pv. tomato* DC3000 exhibits greatly elevated levels of expression of coronatine and coronatine biosynthetic genes. Normal levels of coronatine production can be restored to this *hrcC* mutant by complementation with the gene encoding the *hrp* negative regulator *hrpV,* which lies downstream of *hrcC* in the *hrpC* operon (Deng *et al*., 1998; Penaloza-Vazquez *et al*., 2000). HrpV has been shown to act upstream of *hrpL* expression in the Hrp regulatory cascade (Preston *et al*., 1998b), which suggests that there is cross talk between the Hrp and coronatine regulatory cascades that may be linked to their common regulation by $\sigma^{54}$.

The global regulators GacA and LemA have not yet been characterized in *P. s.* pv. *tomato*, but *gacA* and *lemA* have been shown to regulate a variety of virulence factors, including protease and syringomycin biosynthesis in *P. s.* pv. *syringae* B728a, and *P. s.* pv. *syringae* B728a *gacA* and *lemA* mutants are attenuated in virulence and lesion formation, although a *lemA* mutant of *P. s.* pv. *phaseolicola* does not have a strong phenotype (Hrabak and Willis, 1992, 1993; Rich *et al*., 1994).

Quorum sensing, mediated by low molecular weight diffusable molecules such as N-acyl homoserine lactones (AHLs), is known to regulate a range of metabolic processes in Gram-negative bacteria, including virulence (Bassler, 1999; Parsek and Greenberg, 2000). Although some strains of *P. s.* pv. *tomato* have been shown to produce AHLs, it is not known whether any aspects of virulence and pathogenicity in *P. s.* pv. *tomato* are regulated by quorum sensing. However it is interesting to note that *P. s.* pv. *syringae* strains with mutations in the global regulators *gacA* and *lemA* produce very low levels of AHL (Dumenyo *et al*., 1998).

## FUNCTIONAL GENOMICS, CELL BIOLOGY AND THE FUTURE

The challenge facing researchers studying *P. s.* pv. *tomato* in the future will not only be to determine the factors that account for pathogenesis and virulence in a particular strain on a particular host, but also to determine which of these factors account for the variability in symptom production and host range between strains; to address the consequences of this variability in an agricultural and ecological context; and to exploit the ability of *P. s.* pvs. *tomato* and *maculicola* to infect multiple host species to examine the effect of host species on pathogenesis.

The type III secreted protein AvrE exemplifies the challenge of dissecting gene function in *P. s.* pv. *tomato*. *avrE* homologues are widespread in bacteria carrying group I *hrp* clusters, including *E. amylovora and* the plant growth promoting rhizobacterium *P. fluorescens* (Bogdanove *et al*., 1998; G. Preston, N. Bertrand and P. Rainey, unpublished data), but while the AvrE homologue DspE (DspA) in *E. amylovora* is essential for pathogenesis, an AvrE mutant of *P. s.* pv. *tomato* PT23 is only reduced in virulence, and an AvrE mutation in *P. s.* pv. *tomato* DC3000 has virtually no effect (Bogdanove *et al*., 1998; Gaudriault *et al*., 1997; Lorang and Keen, 1995; Lorang *et al*., 1994). The biochemical function of AvrE remains unclear, as is whether it is active in the apoplast or within plant cells, and although AvrE confers avirulence on soybean, the cognate *R* gene has not been identified. A similar challenge is posed by the phytotoxin coronatine, which appears to be essential for pathogenicity in some strains but only enhances virulence in others (Penaloza-Vazquez *et al*., 2000).

Our current knowledge of the biology of *P. s.* pv. *tomato* can be combined with the potential afforded by recent advances in technology to identify a shortlist of some of the key research areas for the next few years.

- Development of techniques to determine the cellular destination and targets of putative type III secreted effector proteins in the absence of gene-for-gene resistance.
- Further characterization of the physiological effects and mode of action of type III secreted effector proteins and other virulence factors.
- Development of improved techniques to determine the distribution and physiological status of individual bacteria *in planta*.
- Whole genome sequence data for at least one strain of *P. s.* pv. *tomato.*
- Application of functional genomics and proteomics to bacteria *in planta* to identify novel virulence factors and analyse the coordination and significance of multiple factors during different stages of the *P. s.* pv. *tomato* lifecycle.
- Application of functional genomics and proteomics to both resistant and susceptible host plants inoculated with *P. s.* pv. *tomato*.

Research into *P. s.* pv. *tomato* has taken scientists to a deeper understanding of the molecular interactions between pathogen and host than almost any other pathogen, particularly in terms of host resistance and specificity. The next wave of plant pathology research will need to overcome the challenges posed by pathogenesis and host susceptibility—but the evidence suggests that *P. s.* pv. *tomato* is still the right pathogen for the job.

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## REFERENCES


**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.

**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.** (1997) The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, Avr proteins, and death. *J. Bacteriol.* **179**, 5655–5662.

**Alfano, J.R., Kim, H.-S., Delaney, T.P. and Collmer, A.** (1997) Evidence that the *Pseudomonas syringae* pv. *syringae hrp*-linked *hrmA* gene encodes an Avr-like protein that acts in a *hrp*-dependent manner within tobacco cells. *Mol. Plant-Microbe Interact.* **10**, 580–588.

**Anderson, D.M., Fouts, D.E., Collmer, A. and Schneewind, O.** (1999) Reciprocal secretion of proteins by the bacterial type III machines of plant and animal pathogens suggests universal recognition of mRNA targeting signals. *Proc. Natl Acad. Sci. USA*, **96**, 12 839–12 843.

**Bagdache, M., Voelksch, B. and Fritsche, W.** (1990) The production of different phytotoxins by strains of *Pseudomonas syringae* pv. *tomato. Zentralblatt Fuer Mikrobiol.* **145**, 111–119.

**Bassler, B.L.** (1999) How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr. Opin. Microbiol.* **2**, 582–587.

**Beattie, G.A. and Lindow, S.E.** (1995) The secret life of foliar bacterial pathogens on leaves. *Annu. Rev. Phytopathol.* **33**, 145–172.

**Beattie, G.A. and Lindow, S.E.** (1999) Bacterial colonisation of leaves: a spectrum of strategies. *Phytopathology*, **89**, 353–359.

**Bender, C.L., Alarcon-Chaidez, F. and Gross, D.C.** (1999) *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiol. Mol. Biol. Rev.* **63**, 266–292.

**Bender, C.L., Stone, H.E., Sims, J.J. and Cooksey, D.A.** (1987) Reduced pathogen fitness of *Pseudomonas syringae* pv. *tomato* Tn5 mutants defective in coronatine production. *Physiol. Mol. Plant Pathol.* **30**, 273–283.

**Bent, A.F., Innes, R.W., Ecker, J.R. and Staskawicz, B.J.** (1992) Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. *Mol. Plant-Microbe Interact.* **5**, 372–378.

**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.

**Bogdanove, A.J., Kim, J.F., Wei, Z., Kolchinsky, P., Charkowski, A.O., Conlin, A., Collmer, A. and Beer, S.V.** (1998) 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. and Martin, G.B.** (2000) AvrPto-dependent Pto-interacting proteins and AvrPto-interacting proteins in tomato. *Proc. Natl Acad. Sci. USA*, **97**, 8836–8840.

**Budde, I. and Ullrich, M.S.** (2000) Interactions of *Pseudomonas syringae* pv. *glycinea* with host and nonhost plants in relation to temperature and phytotoxin synthesis. *Mol. Plant-Microbe Interact.* **9**, 951–961.

**Chang, J.H., Rathjen, J.P., Bernal, A.J., Staskawicz, B.J. and Michelmore, R.W.** (2000) *avrPto* enhances growth and necrosis caused by *Pseudomonas syringae* pv. *tomato* in tomato lines lacking either Pto or Prf. *Mol. Plant-Microbe Interact.* **13**, 568–571.

**Charkowski, A., Alfano, J.R., Preston, G.M., Yuan, J., He, S.Y. and Collmer, A.** (1998) The *Pseudomonas syringae* pv. *tomato* HrpW protein has domains similar to harpins and pectate lyases and can elicit the plant hypersensitive response and bind pectate. *J. Bacteriol.* **180**, 5211–5217.

**Clerc, A., Manceau, C. and Nesme, X.** (1998) Comparison of randomly amplified polymorphic DNA with amplified fragment length polymorphism to assess genetic diversity and genetic relatedness within genospecies III of *Pseudomonas syringae*. *Appl. Environ. Microbiol.* **64**, 1180–1187.

**Collmer, A., Badel, J.L., Charkowski, A.O., Deng, W.-L., Fouts, D.E., Ramos, A.R., Rehm, A.H., Anderson, D.M., Schneewind, O., van Dijk, K. and Alfano, J.R.** (2000) *Pseudomonas syringae* Hrp type III secretion system and effector proteins. *Proc. Natl Acad. Sci. USA*, **97**, 8770–8777.

**Cornelis, G. and van Gijsegem, F.** (2000) Assembly and function of type III secretion systems. *Annu. Rev. Microbiol.* **54**, in press.

**Cuppels, D.A. and Ainsworth, T.** (1995) Molecular and physiological characterization of *Pseudomonas syringae* pv. *tomato* and *Pseudomonas syringae* pv. *maculicola* strains that produce the phytotoxin coronatine. *Appl. Environ. Microbiol.* **61**, 3530–3536.

**Davis, K.R., Schott, E. and Ausubel, F.M.** (1991) Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* **4**, 477–488.

**Deng, W.-L., Preston, G.M., Collmer, A., Chang, C.-J. and Huang, H.-C.** (1998) Characterization of the *hrpC* and *hrpRS* operons of *Pseudomonas syringae* pathovars syringae, tomato and glycinea and analysis of the ability of *hrpF*, *hrpG*, *hrcC*, *hrpT* and *hrpV* mutants to elicit the hypersensitive response and disease in plants. *J. Bacteriol.* **180**, 4523–4531.

**van Dijk, K., Fouts, D.E., Rehm, A.H., Hill, A.R., Collmer, A. and Alfano, J.R.** (1999) The Avr (effector) proteins HrmA (HopPsyA) and AvrPto are secreted in culture from *Pseudomonas syringae* pathovars via the Hrp (Type III) protein secretion system in a emperature- and pH- sensitive manner. *J. Bacteriol.* **181**, 4790–4797.

**Dumenyo, C.K., Mukherjee, A., Chun, W. and Chatterjee, A.K.** (1998) Genetic and physiological evidence for the production of N-acyl homoserine lactones by *Pseudomonas syringae* pv. *syringae* and other fluorescent plant pathogenic *Pseudomonas* species. *Eur. J. Plant Pathol.* **104**, 569–582.

**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.

**Freiberg, C., Fellay, R., Bairoch, A., Broughton, W.J., Rosenthal, A. and Perret, X.** (1997) Molecular basis of symbiosis between *Rhizobium* and legumes. *Nature*, **387**, 394–491.

**Galan, J.E. and Collmer, A.** (1999) Type III secretion machines: bacterial devices for delivery of proteins into host cells. *Science*, **284**, 1322–1328.

**Gardan, L., Shafik, H., Belouin, S., Broch, R., Grimont, F. and Grimont, P.A.D.** (1999) DNA relatedness among the pathovars of *Pseudomonas syringae* and description of *Pseudomonas tremae* sp. *nov* and *Pseudomonas cannabina* sp. *nov. Int. J. Syst. Bacteriol.* **49**, 469–478.

**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.

**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.

**Gross, M. and Rudolph, K.** (1987) Studies on the extracellular polysaccharides (EPS) produced in vitro by *Pseudomonas phaseolicola*: I. Indications for a polysaccharide resembling alginic acid in seven *Pseudomonas syringae* pathovars. *J. Phytopathol.* **118**, 276–287.

**Ham, J.H., Bauer, D.W., Fouts, D.E. and Collmer, A.** (1998) A cloned *Erwinia chrysanthemi* Hrp (type III protein secretion) system functions in *Escherichia coli* to deliver *Pseudomonas syringae* Avr signals to plant cells and to secrete Avr proteins in culture. *Proc. Natl Acad. Sci. USA*, **95**, 10 206–10 211.

**Hanekamp, T., Kobayashi, D., Hayes, S. and Stayton, M.M.** (1997) Avirulence gene D of *Pseudomonas syringae* pv. *tomato* may have undergone horizontal transfer. *FEBS Lett.* **415**, 40–44.

**Hendrickson, E.L., Guevera, P. and Ausubel, F.M.** (2000a) The alternative sigma factor RpoN is required for *hrp* activity in *Pseudomonas syringae* pv. *maculicola* and acts at the level of *hrpL* transcription. *J. Bacteriol.* **182**, 3508–3516.

**Hendrickson, E.L., Guevera, P., Penaloza-Vazquez, A., Shao, J., Bender, C. and Ausubel, F.M.** (2000b) Virulence of the phytopathogen *Pseudomonas syringae* pv. *maculicola* is *rpoN* dependent. *J. Bacteriol.* **182**, 3498–3507.

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

**Hoyos, M.E., Stanley, C.M., He, S.Y., Pike, S., Pu, X.-A. and Novacky, A.** (1996) The interaction of $Harpin_{Pss}$ with plant cell walls. *Mol. Plant-Microbe Interact.* **9**, 608–616.

**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.

**Hueck, C.J.** (1998) Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* **62**, 379–433.

**Hutcheson, S.W., Pirhonen, M.U., Rowley, D.L., Lidell, M.C., Lee, S.W. and Keen, N.T.** (1996) *Pseudomonas syringae hrp* genes: regulation and role in the phenotypic expression of *avr* genes. In: *Pseudomonas Syringae Pathovars and Related Pathogens*. (Rudolf, K., Burr, T.J., Mansfield, J.W., Stead, D., Vivian, A. and von Kietzell, J., eds). Dordrecht, The Netherlands: Kluwer Academic Publishers.

**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.

**Jackson, R.W., Athanassopoulos, E., Tsiamis, G., Mansfield, J.W., Sesma, A., Arnold, D., Gibbon, M.J., Murillo, J., Taylor, J.D. and Vivian, A.** (1999) Identification of a pathogenicity island, which contains genes for virulence and avirulence, on a large native plasmid in the bean pathogen *Pseudomonas syringae* pathovar *phaseolicola*. *Proc. Natl Acad. Sci. USA*, **96**, 10 875–10 880.

**Ji, C., Boyd, C., Slaymaker, D., Okinaka, Y., Takeuchi, Y., Midland, S.L., Herman, E. and Keen, N.** (1998) Characterization of a 34 kDa soybean binding protein for the syringolide elicitors. *Proc. Natl Acad. Sci. USA*, **17**, 3306–3311.

**Jing, Y., Penaloza-Vazquez, A., Chakrabarty, A.M. and Bender, C.L.** (1999) Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of *Pseudomonas syringae* pv. *syringae*. *Mol. Microbiol.* **33**, 712–720.

**Keen, N.T. and Buzzell, R.I.** (1991) New disease resistance genes in soybean against *Pseudomonas syringae* pv. *glycinea*: evidence that one of them interacts with a bacterial elicitor. *Theor. Appl. Genet.* **81**, 133–138.

**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.

**Keith, L.W., Boyd, C., Keen, N.T. and Partridge, J.E.** (1997) Comparison of *avrD* alleles from *Pseudomonas syringae* pv. *glycinea*. *Mol. Plant-Microbe Interact.* **10**, 416–422.

**Keun, L.C. and Cooksey, D.A.** (1993) Characterization of chromosomal homologs of the plasmid-borne copper resistance operon of *Pseudomonas syringae*. *J. Bacteriol.* **175**, 4492–4498.

**Kim, J.F., Charkowski, A.O., Alfano, J.R., Collmer, A. and Beer, S.V.** (1998) Sequences related to transposable elements and bacteriophages flank avirulence genes of *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* **11**, 1247–1252.

**Kjemtrup, S., Nimchuk, Z. and Dangl, J.L.** (2000) Effector proteins of phytopathogenic bacteria: bifunctional signals in virulence and host recognition. *Curr. Opin. Microbiol.* **3**, 73–78.

**Kobayashi, D.Y., Tamaki, S.J. and Keen, N.T.** (1990) Molecular characterization of avirulence gene D from *Pseudomonas syringae* pv. *tomato*. *Mol. Plant-Microbe Interact.* **3**, 94–102.

**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.

**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.

**Manceau, C. and Horvais, A.** (1997) Assessment of genetic diversity among strains of *Pseudomonas syringae* by PCR-restriction fragment length polymorphism analysis of rRNA operons with special emphasis on *P. syringae* pv. *tomato*. *Appl. Environ. Microbiol.* **63**, 498–505.

**Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Frary, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D.** (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, **262**, 1432–1436.

**McNellis, T.W., Mudgett, M.B., Li, K., Aoyama, T., Horvath, D., Chua, N.-H. and Staskawicz, B.J.** (1998) Glucocorticoid-inducible expression of a bacterial avirulence gene in transgenic *Arabidopsis thaliana* induces hypersensitive cell death. *Plant J.* **14**, 247–258.

**Meinhardt, L.W., Krishnan, H.B., Balatti, P.A. and Pueppke, S.G.** (1993) Molecular cloning and characterization of a *sym* plasmid locus

that regulates cultivar-specific nodulation of soybean by *Rhizobium fredii* USDA257. *Mol. Microbiol.* **9**, 17–29.

**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 elcitors 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.

**Moore, R.A., Starratt, A.N., Ma, S.W., Morris, V.I. and Cuppels, D.A.** (1989) Identification of a chromosomal region required for biosynthesis of the phytotoxin coronatine by *Pseudomonas syringae* pv. *tomato*. *Can J. Microbiol.* **35**, 910–917.

**Mudgett, M.B. and Staskawicz, B.J.** (1999) Characterization of the *Pseudomonas syringae* pv. *tomato* AvrRpt2 protein: demonstration of secretion and processing during bacterial pathogenesis. *Mol. Microbiol.* **32**, 927–941.

**Murillo, J. and Keen, N.T.** (1994) Two native plasmids of *Pseudomonas syringae* pathovar tomato strain PT23 share a large amount of repeated DNA, including replication sequences. *Mol. Microbiol.* **12**, 941–950.

**Murillo, J., Shen, H., Gerhold, D., Sharma, A., Cooksey, D.A. and Keen, N.T.** (1994) Characterization of pPT23B, the plasmid involved in syringolide production by *Pseudomonas syringae* pv. *tomato*. *Plasmid*, **31**, 275–287.

**Nimchuk, Z., Marois, E., Kjemtrup, S., Leister, R.T., Katagiri, F. and Dangl, J.L.** (2000) Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell*, **101**, 353–363.

**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.

**Parsek, M.R. and Greenberg, E.P.** (2000) Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc. Natl Acad. Sci. USA*, **97**, 8789–8793.

**Penaloza-Vazquez, A., Preston, G.M., Collmer, A. and Bender, C.L.** (2000) Regulatory interactions between the Hrp type III protein secretion system and coronatine biosynthesis in *Pseudomonas syringae* pv. *tomato* DC3000. *Microbiology*, **146**, 2447–2456.

**Preston, G.M., Deng, W.-L., Huang, H.-C. and Collmer, A.** (1998b) Negative regulation of *hrp* genes in *Pseudomonas syringae* by HrpV. *J. Bacteriol.* **180**, 4532–4537.

**Preston, G.M., Haubold, B. and Rainey, P.R.** (1998a) Bacterial genomics and adaptation to life on plants: implications for the evolution of pathogenicity and symbiosis. *Curr. Opin. Microbiol.* **1**, 589–597.

**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.

**Rainey, P.B.** (1999) Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. *Env. Microbiol.* **1**, 243–257.

**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.

**Roine, E., Raineri, D.M., Romantschuk, M., Wilson, M. and Nunn, D.N.** (1998b) Characterisation of type IV pilus genes in *Pseudomonas syringae* pv. *tomato* DC3000. *Mol. Plant-Microbe Interact.* **11**, 1048–1056.

**Roine, E., Saarinen, J., Kalkkinen, N. and Romantschuk, M.** (1998a) Purified HrpA of *Pseudomonas syringae* pv. *tomato* DC3000 reassembles into pili. *FEBS Lett.* **417**, 168–172.

**Roine, E., Wei, W., Yuan, J., Nurmiaho-Lassila, E.-L., Kalkkinen, N., Romantschuk, M. and He, S.Y.** (1997) HrpA, a structural protein of a novel bacterial pilus required for plant-bacterial interaction. *Proc. Natl Acad. Sci. USA*, **94**, 3459–3464.

**Ronald, P.C., Salmeron, J.M., Carland, F.M. and Staskawicz, B.J.** (1992) The cloned avirulence gene *avrPto* induces disease resistance in tomato cultivars containing the *Pto* resistance gene. *J. Bacteriol.* **174**, 1604–1611.

**Salmeron, J.M. and Staskawicz, B.J.** (1993) Molecular characterization and *hrp*-dependence of the avirulence gene *avrPto* from *Pseudomonas syringae* pv. tomato. *Mol. Gen. Genet.* **239**, 6–10.

**Scofield, S.R., Tobias, C.M., Rathjen, J.P., Chang, J.H., Lavelle, D.T., Michelmore, R.W. and Staskawicz, B.J.** (1996) Molecular basis of gene-for-gene specificity in bacterial speck disease of tomato. *Science*, **274**, 2063–2065.

**Sesma, A., Sundin, G.W. and Murillo, J.** (1998) Closely related plasmid replicons coexisting in the phytopathogen *Pseudomonas syringae* show a mosaic organization of the replication region and altered incompatibility behaviour. *Appl. Environ. Microbiol.* **64**, 3948–3953.

**Shan, L., He, P., Zhou, J.M. and Tang, X.** (2000) A cluster of mutations disrupt the avirulence but not the virulence function of AvrPto. *Mol. Plant-Microbe Interact.* **13**, 592–598.

**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.

**Taira, S., Tuimala, J., Roine, E., Nurmiaho-Lassila, E.L., Savilahti, H. and Romantschuk, M.** (1999) Mutational analysis of the *Pseudomonas syringae* pv. *tomato* hrpA gene encoding Hrp pilus subunit. *Mol. Microbiol.* **34**, 737–744.

**Tamura, K., Zhu, Y., Sato, M., Teraoka, T., Hosokawa, D. and Watanabe, M.** (1998) Roles of coronatine production by *Pseudomonas syringae* pv. *maculicola* for pathogenicity. *Ann. Phytopathol. Soc. Jpn.* **64**, 299–302.

**Tang, X., Frederick, R.D., Zhou, J., Halterman, D.A., Jia, Y. and Martin, G.B.** (1996) Initiation of plant disease resistance by physical interaction of AvrPto and Pto kinase. *Science*, **274**, 2060–2062.

**Tobias, C.M., Oldroyd, G.E., Chang, J.H. and Staskawicz, B.J.** (1999) Plants expressing the Pto disease resistance gene confer resistance to recombinant PVX containing the avirulence gene AvrPto. *Plant J.* **17**, 41–50.

**Vivian, A. and Gibbon, M.J.** (1997) Avirulence genes in plant-pathogenic bacteria: Signals or weapons? *Microbiology*, **143**, 693–704.

**Vivian, A. and Mansfield, J.** (1993) A proposal for a uniform genetic nomenclature for avirulence genes in phytopathogenic pseudomonads. *Mol. Plant-Microbe Interact.* **6**, 9–10.

**Voelksch, B. and Weingart, H.** (1998) Toxin production by pathovars of *Pseudomonas syringae* and their antagonistic activities against epiphytic microorganisms. *J. Basic Microbiol.* **38**, 135–145.

**Wei, W., Plovanich-Jones, A., Deng, W.-L., Ling, J.Q., Collmer, A., Huang, H.-C. and He, S.Y.** (2000) The gene coding for the Hrp pilus structural protein is required for type III secretion of Hrp and Avr proteins in *Pseudomonas syringae* pv. *Tomato. Proc. Natl Acad. Sci. USA*, **97**, 2247–2252.

**Whalen, M.C., Innes, R.W., Bent, A.F. and Staskawicz, B.J.** (1991) Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. *Plant Cell*, **3**, 49–59.

**White, F.F., Yang, B. and Johnson, L.B.** (2000) Prospects for understanding avirulence gene function. *Curr. Opin. Plant. Biol.* **3**, 291–298.

**Wiebe, W.L. and Campbell, R.N.** (1993) Characterization of *Pseudomonas syringae* pv. *maculicola* and comparison with *Pseudomonas syringae* pv. *tomato*. *Plant Dis.* **77**, 414–419.

**Yucel, I., Boyd, C., Debnam, Q. and Keen, N.T.** (1994a) Two different classes of *avrD* alleles occur in pathovars of *Pseudomonas syringae*. *Mol. Plant-Microbe Interact.* **7**, 131–139.

**Yucel, I., Midland, S.L., Sims, J.J. and Keen, N.T.** (1994b) Class I and class II avrD alleles direct the production of different products in gram-negative bacteria. *Mol. Plant-Microbe Interact.* **7**, 148–150.