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; Pseudomonadales 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.


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).

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 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. SYRINGAE GENOSPECIES III AND CAUSE DISEASES OF TOMATOES AND CRUCIFERS**

P. s. pv. 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 pathogens attributed to genomospecies III include *P. primulae*, *P. viburni*, *P. aphidis*, *P. delphinii*, *P. passeriflorae*, *P. philadelphii*, and *P. 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 pathogens 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 asymptptomatically 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 defense 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 *hrp* 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., 1998; 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 Dijck et al., 1999).
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. syringae 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.

(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. syringae 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 non-host 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 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 *hp* 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

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**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 Hpl regulated promoters (based on Alfano et al. (2000) and Collmer et al. (2000)). (B) Model of Hr secretion in *P. s. pv. tomato* showing putative destinations and biological activities of Avr proteins (black circles) and type III accessory proteins (as labeled). 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)
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. Harpins are thought to act as accessory proteins for type III secretion.
domain that has pectate-binding activity (Charkowski et al., 1998). HrpZ does not have a recognizable pectate-binding domain, but HrpZ from P. syringae has been shown to interact with plant cell walls (Heyos 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 an 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 genetically 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, Xie 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 AvrRpt2 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 RPM2 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 (Nimmich 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 phytoxin coronatine (COR) and coronatine derivatives, but a phaseolotoxin-like substance has also been characterized from some P. s. pv. tomato strains (Bagache et al., 1990), and certain strains of P. s. pv. tomato have been shown to have antimicrobial activity (Voelkhch 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 (Buddle 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; Penaloz-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 P. s. pv. maculicola strain are plasmid-borne, as are the 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 cor 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; Penaloz-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 secretion 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 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 T30 kb coronatine biosynthesis cluster and has been shown to produce coronatine genes in *P. s. pv. tomato* DC3000 abolish symptom expression following the spray or dip inoculation of tomato leaves and greatly reduce multiplication in planta (Mittal and Davis, 1995; Penaloz-Vazquez et al., 2000). Internal to plasmid pPT23A is a 24-kb region that includes the avrD gene and its homologues of the UV resistance genes rulAB.

(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 IS1240 at the 5′ region of *P. s. pv. tomato* avrD (Hanekamp et al., 1997), and a Tn501 sequence flanking *avrRpm1* and its homologue from *P. s. pv. pisi* *avrPsiA1*. *avrRpo* 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 (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 major 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., 1998).

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, σ^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 genes. Normal levels of coronatine production can be restored to this hrcC mutant by complementation with the gene encoding

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 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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Pseudomonas syringae pv. tomato


