42 Chaveroche, M.K. *et al.* (2000) A rapid method for efficient gene replacement in the filamentous fungus *Aspergillus nidulans*. *Nucleic Acids Res.* 28, E97
43 Brown, J.S. and Holden, D.W. (1998) Insertional mutagenesis of pathogenic fungi. *Curr. Opin. Microbiol.* 1, 390–394
44 de Groot, M.J. *et al.* (1998) *Agrobacterium tumefaciens*-mediated transformation of filamentous fungi. *Nat. Biotechnol.* 16, 839–842
45 Magrini, V. and Goldman, W.E. (2001) Molecular mycology: a genetic toolbox for *Histoplasma capsulatum*. *Trends Microbiol.* 9, 541–546
46 Li-Destri-Nicosia, M.G. *et al.* (2001) Heterologous transposition in *Aspergillus nidulans*. *Mol. Microbiol.* 39, 1330–1344
47 Villalba, F. *et al.* (2001) Transposon *impala*, a novel tool for gene tagging in the rice blast fungus *Magnaporthe grisea*. *Mol. Plant Microbe Interact.* 14, 308–315
48 Hua-Van, A. *et al.* (2001) Transposition of autonomous and engineered *impala* transposons in *Fusarium oxysporum* and a related species. *Mol. Gen. Genet.* 264, 724–731
49 Craig, N.L. (1997) Target site selection in transposition. *Annu. Rev. Biochem.* 66, 437–474
50 De Backer, M.D. *et al.* (2002) RNA-mediated gene silencing in non-pathogenic and pathogenic fungi. *Curr. Opin. Microbiol.* 5, 323–329
51 De Backer, M.D. *et al.* (2001) An antisense-based functional genomics approach for identification of genes critical for growth of *Candida albicans*. *Nat. Biotechnol.* 19, 235–241
52 Gorlach, J.M. *et al.* (2002) Antisense repression in *Cryptococcus neoformans* as a laboratory tool and potential antifungal strategy. *Microbiology* 148, 213–219
53 Hammond, S.M. *et al.* (2001) Post-transcriptional gene silencing by double-stranded RNA. *Nat. Rev. Genet.* 2, 110–119
54 Hannon, G.J. (2002) RNA interference. *Nature* 418, 244–251
55 Liu, H. *et al.* (2002) RNA interference in the pathogenic fungus *Cryptococcus neoformans*. *Genetics* 160, 463–470

Genomic mining type III secretion system effectors in *Pseudomonas syringae* yields new picks for all TTSS prospectors

Alan Collmer, Magdalen Lindeberg, Tanja Petnicki-Ocwieja, David J. Schneider and James R. Alfano

Many bacterial pathogens of plants and animals use a type III secretion system (TTSS) to deliver virulence effector proteins into host cells. Because effectors are heterogeneous in sequence and function, there has not been a systematic way to identify the genes encoding them in pathogen genomes, and our current inventories are probably incomplete. A pre-closure draft sequence of *Pseudomonas syringae* pv. *tomato* DC3000, a pathogen of tomato and *Arabidopsis*, has recently supported five complementary studies which, collectively, identify 36 TTSS-secreted proteins and many more candidate effectors in this strain. These studies demonstrate the advantages of combining experimental and computational approaches, and they yield new insights into TTSS effectors and virulence regulation in *P. syringae*, potential effector targeting signals in all TTSS-dependent pathogens, and strategies for finding TTSS effectors in other bacteria that have sequenced genomes.

Published online: 06 September 2002

Extracellular proteins promote the virulence of bacterial pathogens of both plants and animals, and 'effector' proteins injected into host cells by type III secretion systems (TTSSs) are particularly important in the virulence of Gram-negative bacteria. TTSSs use a highly conserved machinery to secrete proteins through the bacterial envelope and a more variable extracellular machinery to translocate proteins through host barriers such as the plant cell wall and plasma membrane. The structure, assembly and regulation of TTSSs have recently been reviewed [1–4].

Pathogens of animals that use TTSSs include *Yersinia*, *Salmonella* and *Shigella* spp., *Pseudomonas aeruginosa* and enteropathogenic *Escherichia coli*. Many effectors in these pathogens, such as the six known *Yersinia* outer protein (Yop) effectors, target the host cytoskeletal and defense systems [5]. Pathogens of plants that use a TTSS include *Pseudomonas syringae*, *Ralstonia solanacearum* and several *Xanthomonas*, *Erwinia* and *Pantoea* spp. The TTSS in these pathogens is known as the hypersensitive response and pathogenicity (Hrp) system because secretion mutants are unable to elicit the defense-associated hypersensitive response in non-host plants or establish a pathogenic (or parasitic) relationship with hosts. TTSS substrates in *P. syringae* are known primarily as Avrs (for the avirulence they can confer in test plants) or Hops (Hrp outer proteins) (see Box 1 for an explanation of the nomenclature used for *P. syringae* TTSS substrates). As we will discuss, some Hops might be components of the translocator rather than true effectors, and here we will use 'effector' as suggested by Cornelis and Van Gijsegem [3] to denote the subset of TTSS-secreted proteins that function primarily inside the host cell. How phytopathogenic TTSS effectors

Box 1. Decoding the multiple names for *Pseudomonas syringae* TTSS substrates

The names of *P. syringae* proteins secreted by the Hrp system reflect the phenotype, source strain and priority, as explained below:
Hop (Hrp outer protein): Applies generically to all proteins secreted or translocated by the Hrp system of *P. syringae* and other plant pathogens with similar Hrp systems (e.g. *Erwinia* and *Pantoea* spp). For example, $HopPmaJ_{Pto}$ denotes the *P. s. tomato* homolog of a Hop first demonstrated to be translocated in *P. s. maculicola* [a].
Avr (avirulence protein): Denotes a protein found through the avirulence phenotype it confers on a *P. syringae* strain in appropriate test plants. For example, $AvrPpiC2_{Pto}$ is the *P. s. tomato* homolog of an Avr protein first found in *P. s. pisi*. Note, the *P. s. tomato* homolog has now been renamed HopPtoC because a Hop (secretion) phenotype has been demonstrated, whereas an Avr phenotype has not [b].
Hrp (hypersensitive response and pathogenicity): Most mutations in the TTSS machinery abolish the ability of *P. syringae* to elicit the hypersensitive response in non-hosts or to be pathogenic in hosts. HrpA (the Hrp pilus subunit) represents a TTSS substrate that retains its original designation because of its Hrp phenotype and/or association with the Hrp system.
Effector: A term for virulence proteins injected into host cells by a TTSS that is broadly applicable to various pathogens of plants and animals.
Helper protein: A term of convenience used in this review to encompass extracellular accessory proteins (such as HrpA) plus other TTSS substrates (such as harpins) whose primary function is likely to be the translocation of true effectors through host barriers.
Harpins: Presumed helper proteins that are secreted by the TTSS in more abundance than true effectors, appear to interact with plant cell walls and membranes, are glycine-rich and devoid of cysteine, and possess a heat-stable ability to elicit the hypersensitive response when infiltrated into the intercellular (apoplastic) spaces of plant leaves.

References

a 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
b 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

promote parasitism is unknown, although recent papers reveal that a subset have biologically active cysteine protease catalytic triads like those found in YopJ and YopT, which suggests they have similar functions in the subversion of host defenses [6,7]. The binding of AvrRpm1 to the *Arabidopsis* RIN4 protein, a potential regulator of basal plant defenses, further supports the idea that effector proteins function in suppressing plant defenses [8].

A striking difference between the TTSS effectors of plant and animal pathogens is the abundance with which they are secreted in culture. For example, Yops are readily detected in the media of *Yersinia* cultured without Ca^{2+} at 37°C, whereas many plant pathogen TTSS effectors are barely detectable in culture media, even with sensitive immunodetection [9–11]. Thus, identifying effector genes has been a challenge since they were first discovered as 'avirulence' loci a decade before the discovery of the TTSS [12]. Table 1 summarizes approaches and problems encountered in the search for effector genes before the genomics era. Because of the weak contribution of individual effector genes to virulence (apparently owing to redundancy), they are not detected in mutant screens. Further problems are the happenstance basis for avirulence phenotypes (dependent on the 'luck' of a test plant carrying an *R* gene that will direct recognition of the candidate effector), and the lack of any conserved structural features (most plant pathogen effectors appear unrelated and highly divergent). Consequently, only a single TTSS effector (AvrE) had been characterized at a molecular level in the model pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (which causes bacterial speck of tomato and *Arabidopsis*) before sequence-driven analyses began in 2000 [13]. The public availability of a pre-closure draft sequence of the DC3000 genome (http://www.tigr.org/tdb/mdb/mdbinprogress.html) has now fostered a new attack on the inventory of effectors and other TTSS substrates, and here we discuss five papers that, collectively, yield a first look at the DC3000 effector inventory and demonstrate the application of several tools that could be useful for mining effector genes in other pathogens using type III secretion.

Table 1. Methods used to identify TTSS effectors in plant pathogens before the availability of sequenced genomes[a]

Method	Comment	Refs
Avirulence phenotype	Heterologous expression of a pathogen cosmid library in virulent recipient reveals presence of *avr* gene when HR is triggered in inoculated test plants; source of most previously known Hrp effectors	[12,60, 61]
Presence in pathogenicity islands	Most plant pathogen Hrp TTSS gene clusters are flanked by several effector genes; other effector genes have been found in pathogenicity islands elsewhere on plasmids or chromosomes	[21,52, 62]
Antibodies against proteins secreted by TTSS in culture	This approach appears to yield the more abundantly secreted translocation helper components	[38]
cDNA-AFLP	Has yielded candidate effectors in the Hrp regulon of *Xanthomonas campestris* pv. *vesicatoria*	[63]

[a]Abbreviations: AFLP, amplified fragment-length polymorphism; HR, hypersensitive response; TTSS, type III secretion systems.

Features associated with TTSS substrates that can be used in genomic mining

Despite the highly variable structure and function of *P. syringae* effectors, their coordinated regulation and ability to be secreted or translocated by a TTSS are conserved features that can be exploited for genome-wide searches (Fig. 1). Several features of the Hrp system are notable here. Induction in plant apoplastic fluids or apoplast-mimicking media is activated by the σ^{54}-enhancer-binding proteins HrpR and HrpS [14–16], which in turn activate the RpoN-dependent expression of alternate sigma factor HrpL [17]. HrpL is believed to bind to 'Hrp box' promoter sequences located upstream of genes in the Hrp regulon, namely the TTSS machinery and all the effector genes that have been found to date through avirulence phenotypes [18–20]. Sequencing of the *hrp* region of *P. s. tomato* DC3000 and *P. s. syringae* strains 61 and B728a revealed that the genes encoding the TTSS machinery are at the center of a tripartite pathogenicity island that includes exchangeable and conserved effector loci containing *avrE* and perhaps five other effector genes [21]. All of these candidate effector genes are also preceded by Hrp box promoters, which further encourages searching based on linkage with these sequences.

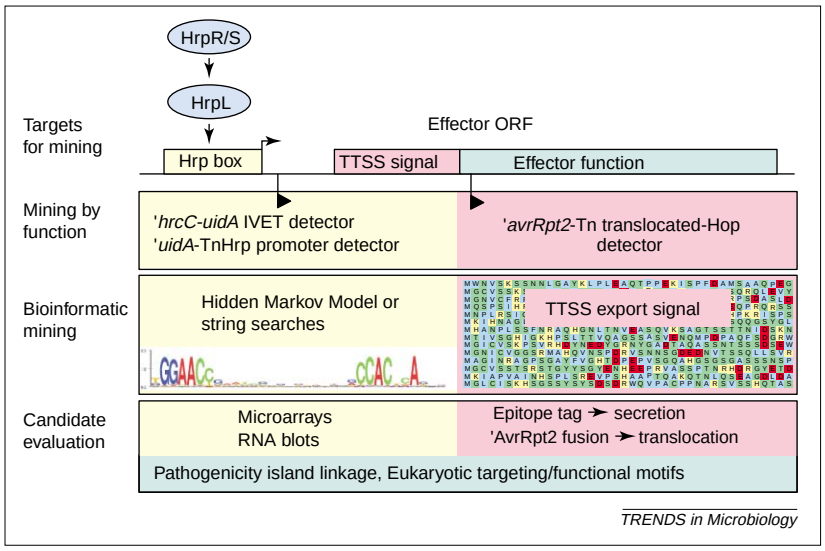

Fig. 1. Mining for Hrp type III secretion system (TTSS) effectors based on function or on patterns in HrpL-responsive promoters and in TTSS signal regions. 'Hrp box' promoters are activated by the HrpR/S-HrpL cascade and express effectors, which characteristically have an amino-terminal TTSS signal region and a larger carboxy-terminal effector function region. Hrp promoters in the genome can be functionally identified by HrpL activation of genes found through reporter transposon insertions or *in vivo* expression technology (IVET). A sample set of functional Hrp promoters can be used to construct a Hidden Markov Model or string search for bioinformatic mining of the entire genome. Candidate Hrp promoters can then be evaluated for function by analysis with RNA blots or microarrays (as yet not whole-genome) and used to refine further the bioinformatic search tools. Independent of regulation, effector genes can be identified by *'avrRpt2*-Tn insertions: a diagnostic plant response indicates fusion with a protein carrying TTSS signals. Alternatively, bioinformatic mining can be carried out using putative TTSS export signals. The TTSS-dependent secretion or translocation of candidate effectors can then be tested by immunoblotting or with 'AvrRpt2 fusions. Additional factors in evaluating candidates include linkage with other effectors, signatures of horizontal transfer and the presence of motifs implicated in subcellular targeting or function within eukaryotic cells.

Several features of the Hrp secretion system also suggest possibilities for new tools that could be used for effector mining. TTSS effector proteins appear to have a modular structure, with the amino-terminal region (1–150 amino acids) carrying targeting information and the remainder of the protein directing effector activity [3]. This finding supports the use of reporter fusions for detecting secretion or translocation. For example, hybrid proteins comprising the amino-terminal region of a putative Hop and the effector domain of AvrRpt2 can elicit an *RPS2*-dependent hypersensitive response (an indicator of translocation into the plant cytoplasm) when delivered by Hrp TTSS-proficient bacteria into appropriate test plants [22]. The first 15 codons or amino acids of effectors are particularly important in TTSS secretion, and this secretion signal appears to function universally. For example, Yops and Hops can be heterologously secreted by Ysc and Hrp TTSS pathways [23]. However, the nature of the targeting signal(s) is controversial [24]. Amphipathicity is clearly important [25], but this is too general a property to use in genome-wide searches for novel effector genes. Nevertheless, the portability and relatively small size of the targeting region encourages the development of new methods of searching for effectors. As depicted in Fig. 1 and described below, regulation and export signals have been exploited in both bioinformatic and functional assays to find and confirm novel Hops in *P. s. tomato* DC3000.

Alan Collmer
Magdalen Lindeberg
Dept of Plant Pathology, Cornell University, Ithaca, NY 14850-4203, USA.

David J. Schneider
United States Dept of Agriculture-Agricultural Research Service Center for Agricultural Bioinformatics, Cornell Theory Center, Cornell University, Ithaca, NY 14853-2901, USA.

Tanja Petnicki-Ocwieja
James R. Alfano*
Plant Science Initiative and Dept of Plant Pathology, University of Nebraska, Lincoln, NE 68588-0660, USA.
*e-mail: jalfano2@unl.edu

Identifying candidate TTSS effector genes in *P. syringae* based on regulation

Three of the five recent papers exploiting the *P. s. tomato* DC3000 draft sequence identify novel virulence genes based on expression *in planta* or on membership of the Hrp regulon. *In vivo* expression technology (IVET; pioneered in 1987 with the plant pathogen *Xanthomonas campestris* pv. *campestris* [26]) was used by Boch *et al.* [27] to identify promoters that could restore *hrcC* expression to *P. s. tomato* DC3000 *in planta*. Of the 69 *in planta*-expressed (*ipx*) genes that were initially analyzed (from a pool of 268 unique *ipx* strains), 22 have promoter regions containing well-conserved Hrp boxes and most of these exhibit HrpL-dependent expression in *E. coli*. This reveals that the Hrp regulon contains a large subset of the genes that are expressed during infection. As expected, several of the *ipx* fusions are to genes that were revealed by further analysis to be *hop*/*avr* genes (Table 2).

Degeneracy in known Hrp box sequences is an obstacle to using bioinformatics to identify Hrp promoters in the *P. syringae* genome. Fouts *et al.* [28] addressed this problem by using a reporter transposon to identify a sample set of HrpL-activated DC3000 promoters and then the enlarged set of functional Hrp promoters to train a Hidden Markov Model (HMM) for genome-wide mining. Alternatively, Zwiesler-Vollick *et al.* [29] used a string search that accommodated common variations in Hrp promoters to identify putative Hrp promoters. Subsequent microarray and RNA-blot analyses confirmed the HrpL- or HrpS-dependent expression of many of the promoters found by the HMM and string search methods, and several candidate *hop* genes with similarities to known *avr*/*hops* were identified downstream of these newly found Hrp promoters. These data indicate that: there are probably 50–100 well-activated Hrp promoters in DC3000; a subset of ORFs downstream of Hrp promoters appear unrelated to the TTSS/effector system; the Hrp regulon controls other virulence-related regulons (such as the coronatine toxin biosynthesis genes); and many of the ORFs downstream of Hrp promoters have no homologs in the databases. Most of these unknown ORFs show signatures of horizontal acquisition that are typical of effector genes, and many were subsequently shown to possess features associated with TTSS substrates and to travel the Hrp TTSS pathway (Table 2).

Identifying candidate TTSS effector genes in *P. syringae* based on secretion and translocation

Two of the recent papers exploring the *P. s. tomato* DC3000 genome identify novel effectors on the basis of their secretion or translocation abilities and sequence features predictive of TTSS substrates.

Table 2. Characteristics of Hop, Avr and candidate Hop proteins identified in *P. syringae* pv. *tomato* DC3000

Pto DC3000 Hops[a]	Amino terminal export signal[b]	% Ser[c]	Hop expression in DC3000: IVET[d]	Hop expression in DC3000: Hrp-dependence[e]	Hop secretion/ translocation[f]	Gene locale[g]	Likely function and/or homolog[h] (BLASTP E value; GenBank accession no.)	Refs
Helper proteins								
HopPmaG[i]$_{Pto}$	–	18	–	H, T	ND	C	Transglycosylase (1E-106; NP_643533)	[30,31]
HopPmaH$_{Pto}$ (Pectin lyase-related)	+	6	+	H, M, I, T	ND	C	Pectin lyase (1E-54; BAA12119)	[30,31]
HopPtoP (HrpW-related)	+	20	–	H, M	T	C	HrpW (3E-04; AF232004)	[28,31]
HrpA$_{Pto}$	+	8	+	H, I, M, R	S	C	Hrp pilus	[32]
HrpK$_{Pto}$	+	8	+	H, I, M	S[j]	C	NA	[21]
HrpW$_{Pto}$	+	12	–	H, M, R, T	S	C	Harpin/pectate lyase	[37]
HrpZ$_{Pto}$	+	24	–	H	S	C	Harpin	[38,64, 65]
Effectors								
AvrE$_{Pto}$	+	16	–	H, M, R	ND	C	NA	[13]
AvrPphE$_{Pto}$	+	14	–	H, M, T	ND	P	AvrPphE (1e-117; AAA67930)	[28]
AvrPpiB1$_{Pto}$	+	16	–[k]	H, M	ND	C	AvrPpiB1 (1e-152; S57627)	[28]
AvrPpiB2$_{Pto}$	+	16	+[k]	H, I, M	ND	P	AvrPpiB1 (1e-152; S57627)	[28]
AvrPto$_{DC3000}$	+	16	–	H, M, R, T	S	C	AvrPto$_{JL1065}$ (3e-79; S35220)	[10,28]
AvrPtoB	+	18	+	H, I, M, R, T	T	C	VirPphA (7e-95; AAD47203)	[66]
HopPmaI$_{Pto}$	+	14	+	H, I, M, R, T	ND	C	DnaJ domain (2E-11; BAB17689)	[30,31]
HopPmaJ$_{Pto}$	+	12	–	NH	ND	C	HopPmaJ (7e-65; AF458048)	[31]
HopPsyA$_{Pto}$	+	10	–	H, M, R, T	ND	C	HopPsyA (1e-92; AF232003)	[28]
HopPtoA1 (CEL ORF5)	+	18	–	H, M, T	S	C	HopPtoA2 (1e-177; AF469470)	[67]
HopPtoA2	+	20	+	H	S	C	HopPtoA1 (1e-177; AF232004)	[67]
HopPtoB1 (EEL ORF1)	+	12	–	H	S[j]	C	NA	[21]
HopPtoC (AvrPpiC2$_{Pto}$)	+	16	–	H, T, M, R	S	C	Cysteine protease; AvrPpiC2 (1e-148; CAC16701)	[7,30]
HopPtoD1[l] (AvrPphD1$_{Pto}$)	+	14	–	H, M, R	S	C	AvrPphD (0.0; CAC16699)	[30]
HopPtoD2[l] (AvrPphD2$_{Pto}$)	+	20	–	H, M, R	S	C	AvrPphD (3e-44; CAC16699)	[30]
HopPtoE[m]	+	14	+	H, I, M, R	S, T	C	NA	[29,30]
HopPtoF (AvrPphFORF2$_{Pto}$)	+	20	–	H, M, R	T[n]	C	HR suppressor; AvrPphF ORF2 (2e-44; AF231452)	[28,53]
HopPtoG	+	10	–	H	S, T	C	*R. solanacearum* hypothetical protein (1e-137; NP_521884)	[30]
HopPtoH	+	10	–	H	S	C	*P. s. pisi* ORF3 (1e-114; CAC16702)	[30]
HopPtoI	+	16	–	H	S	C	NA	[30]
HopPtoJ (AvrXv3$_{Pto}$)	+	26	–	H	S	C	AvrXv3 (1e-11; AAG18480)	[30]
HopPtoK (AvrRps4$_{Pto}$)	+	18	–	H	T	C	AvrRps4 (2e-44; AAB51082)	[30]
HopPtoL	+	4	–	NH	S	C	SrfC (1E-21; AAF74575)	[30]
HopPtoM (CEL ORF3)	–	10	+	H, I	S[o]	C	NA	[21]
HopPtoN (CEL ORF7)	+	14	+	H, I, M, T	S[o]	C	Cysteine protease	[7,28]
HopPtoS1 (HopPtoO)	+	24	–	H, M, R	S, T	P	Chicken ADP ribosyltransferase (1E-05; P55807)	[30,31]
HopPtoS2	+	10	–	H	S	C	*Clostridium* ADP ribosyltransferase (1E-05; NP_346979)	[30]
HopPtoS3	+	28	–	NH	S[j]	C	Chicken ADP ribosyltransferase (1E-03; P55807)	[30]
HopPtoT1 (ORF16)	+	14	–	H	T[j]	P	HolPtoU2 (1e-156)	[30]

Petnicki-Ocwieja *et al.* used secretion and translocation assays to demonstrate that ten ORFs downstream of HMM-identified Hrp promoters were Hops, and this initial set of confirmed Hops was combined with 18 other established Avr/Hops to identify export signal patterns in the first 50 amino acids of TTSS substrates from *P. syringae* [30]. All possessed a pattern of equivalent solvent-exposed amino acids in the amino-terminal five positions, a lack of Asp or Glu residues in the first 12 positions, and several characteristic properties of the first 50 amino acids, including amphipathicity and richness in Ser and Gln. The six predictive 'rules' describing these features were notably violated by several Hrp-regulated proteins known not to be TTSS substrates. These rules were used to search a draft sequence of the DC3000 genome, independent of Hrp promoters, to yield 129 candidate novel effector ORFs, 32 of which had additional features associated with effectors; the first two tested were confirmed to be Hops [30].

Guttmann *et al.* [31] cleverly used a transposon that generates translational fusions with a truncated AvrRpt2 lacking native TTSS signals to identify 13 Hops in *Pseudomonas syringae* pv. *maculicola*

Table 2. Continued

Pto DC3000 Hops[a]	Amino terminal export signal[b]	% Ser[c]	Hop expression in DC3000: IVET[d]	Hop expression in DC3000: Hrp-dependence[e]	Hop secretion/ translocation[f]	Gene locale[g]	Likely function and/or homolog[h] (BLASTP E value; GenBank accession no.)	Refs
Candidate Hops								
HolPtoAA	+	16	–	H	ND	C	AvrRps4 (5e-6; AAB51082)	[31]
HolPtoR	+	18	–	H, T	ND	C	*R. solanacearum* hypothetical protein (2e-74; NP_522840.1)	[31]
HolPtoU2	–	12	–	NH	ND	C	HopPtoT1 (1e-156)	[31]
HopPtoB2	–	12	–	H	ND	C	HopPtoB1 (2e-16; AF232004)	[28]
Ipx47	+	6	+	NH	ND	C	*Streptomyces coelicolor* putative carboxylase (4e-8; NP_630967)	[27]
Ipx53	+	10	+	I, NH	ND	C	NA	[27]
ORF11 (HrpA-related)	+	16	–	H, M, T	ND	C	HrpA (6e-7; AF232004)	[28]
ORF15	–	14	–	H	ND	C	ORF26 (4e-9)	[30]
ORF19	+	24	–	H	ND	C	*R. solanacearum* hypothetical protein (2e-92; NP_518366)	[30,31]
ORF20	+	8	–	H	ND	C	*X. a. citri* hypothetical protein (3e-9; NP_642586)	[30]
ORF23	+	8	–	H	ND	C	Riboflavin-specific deaminase RibD (8e-38; NP_213307)	[30]
ORF24	–	10	+	H	ND	C	*Clostridium* putative phosphatase (5e-32; NP_347269)	[30]
ORF26	+	24	+	H, I, M, R, T	ND	C	ORF15 (4e-9)	[30,31]
ORF28	+	12	–	H	ND	C	NA	[30]
ORF01152	+	8	–	H, T	ND	C	NA	[28]

[a]Hop designations follow previous recommendations [68] and are given priority over Avr designations in cases where secretion or translocation, but not avirulence, has been demonstrated for the DC3000 homolog. Several Hops in the helper class retain their original Hrp designations.
[b]Indicates whether amino-terminal 50 amino acids possess putative export signal patterns [30].
[c]Percentage of serine in the first 50 amino acids of each protein is indicated.
[d]ORFs that were induced *in planta* using *in vivo* expression technology (IVET) are indicated [27].
[e]Data supporting Hrp-dependent expression are indicated as follows: GUS activity of miniTn*5gus* insertions (T) [28], microarray analyses (M) [28,29], RNA blot analyses (R) [28,29] or HrpL-dependent expression of *ipx* fusions in *E. coli* (I) [27]. Presence (H) or absence (NH) of a Hrp box is also indicated.
[f]Status of DC3000 type III secretion system (TTSS) substrate testing is indicated by S (secretion shown), T (translocation shown) or ND (not done).
[g]Indicates whether the ORF is on a plasmid (P) or in the chromosome (C).
[h]The likely function and any homologs in the databases are given, or if none are known, NA indicates not applicable. The similarities of the indicated TTSS substrates to ADP ribosyltransferases and cysteine proteases were further supported by conserved catalytic sites [7,69].
[i]HopPmaG$_{Pto}$ was reported to be translocated into plant cells although its sequence suggests a function within the bacterial envelope.
[j]Unpublished results from the Alfano laboratory.
[k]The IVET screen identified an *avrPpiB1* allele, but from the published report [27] it is not possible to determine which one of the two alleles was identified.
[l]The IVET screen did not identify either HopPtoD1 or HopPtoD2, however, it identified a third, non-functional allele of *avrPphD* [27].
[m]HopPtoE is referred to as Chp1 in [29].
[n]Xiaoyan Tang, unpublished.
[o]Unpublished results from the Collmer laboratory.

ES4326. Noting that the first 50 amino acids of these proteins had significantly higher Ser and lower Asp contents than the proteins not secreted by the TTSS, they searched the draft sequence of the *P. s. tomato* DC3000 genome for ORFs downstream of Hrp promoter sequences with such amino acid biases. Two of these DC3000 proteins were tested with ′AvrRpt2 fusions and shown to be Hops.

Table 2 summarizes the data from the five papers we have described here and shows the current inventory of *P. s. tomato* proteins that have either been demonstrated to travel the Hrp TTSS pathway or are predicted to do so by multiple criteria that represent the overlap between search methods. A typical candidate Hop is ORF26 (whose complete sequence is available under this designation [30]), which possesses amino-terminal export signals, a high Ser content in the first 50 amino acids, and an Hrp promoter that is expressed *in planta* and is activated by HrpL. The current inventory contains 36 Hops that have been experimentally confirmed by secretion, translocation or avirulence activity in DC3000 (or, in some cases, through functional data for a homolog in another *P. syringae* strain). There are 15 strongly predicted candidate Hops and many more weaker candidates that await functional testing.

Additional Hop candidates might have been overlooked owing to defects in the current mining tools. This possibility is suggested by the existence of atypical Hops such as HopPtoM (no obvious targeting signals), HrpA (only 8% Ser) and HopPtoL (lacking a Hrp promoter and not reported in the IVET screen). More Hops will probably emerge from (1) secretion or translocation testing of all ORFs that have at least one of the features characteristic of Hops; (2) refined genome-wide searches for Hrp promoters and export

signals; and (3) bioinformatic and functional analysis of all ORFs in putative pathogenicity islands that become detectable following closure and annotation of the DC3000 genome. Because the *P. s. tomato* DC3000 effector inventory in Table 2 is likely to continue growing, updated versions will be made available at http://pseudomonas-syringae.org

Hop function: helpers versus effectors

One distinction emerging from analyses of the growing pool of Hops is that proteins secreted by the Hrp pathway appear to fall into two functional categories – 'helpers' and effectors – with the former proteins aiding the delivery of the effectors (Table 2). HrpA is the clearest example of a helper because it is essential for effector delivery and forms the Hrp pilus through which HrpZ and AvrPto were shown to travel [32–34]. Harpins might also assist in effector delivery through interactions with the plant cell wall and plasma membrane: HrpZ interacts with both of these translocation barriers [35,36], and HrpW possesses a carboxy-terminal pectate lyase domain and binds pectate, which is a major component of plant cell walls [37]. HrpA, HrpZ and HrpW are secreted more abundantly than any of the effectors, and the two harpins are unique among known Hops in eliciting the hypersensitive response when exogenously applied to the exterior of plant cells [38]. HopPtoP, $HopPmaH_{Pto}$ and ORF11 are candidate helper Hops because of their similarity to pectin lyase, HrpW and HrpA, respectively, and other properties such as the characteristic lack of any Cys in harpins. HrpK could be a translocator helper based on its weak similarity with the *Xanthomonas campestris* pv. *vesicatoria* HrpF putative translocator protein [39] and mutant analyses suggesting a role in effector delivery (J.R. Alfano, unpublished). The observation that HrpW, HopPmaH and HopPtoP appear capable of targeting AvrRpt2 into the plant cell would at first glance contradict a cell wall-associated 'helper' role for the harpins [31]. However, additional targeting signals within the AvrRpt2 moiety might be capable of directing translocation to the plant cell interior if the fusion partner provides signals for entering the pathway. Alternatively, the fusion might disrupt signals that would normally sort proteins to the apoplast rather than the host cell interior, or the putative helper proteins might have additional activities inside host cells analogous to *Salmonella* SipB [40]. It is also noteworthy here that a HopPmaG-′AvrRpt2 fusion was translocated into plant cells by *P. s. maculicola* [31], although the similarity of HopPmaG to enzymes targeting peptidoglycan suggests a bacterial site of action for this protein. Distinguishing between true effectors, transported into the plant cell, and extracellular helpers might require use of fusion proteins such as CyaA (a reporter used to identify proteins located within eukaryote cells) that are not themselves substrates for TTSS secretion [41,42].

Examining the *P. s. tomato* DC3000 effector inventory for insights into effector function

Now that we have identified many Hops from *P. syringae*, what are their roles in pathogenesis? We have noted the recent clues about effector targets in plants (for reviews see [43–45]). Additional insights can be gleaned from the *P. s. tomato* effector inventory shown in Table 2. Unexpectedly, most of the *hop* genes are chromosomally encoded, with only four being carried on plasmids. Most striking is the prevalence of Hops with similarities to ADP ribosyltransferases [30,31]. Although the similarities between these proteins are limited, the ART domains are conserved and these represent a new class of plant pathogen effectors with a clear potential to modify plant signal transduction pathways. Furthermore, HopPtoS1 and HopPtoS3 have homologs in *R. solanacearum* and *Xanthomonas axonopodis* pv. *citri*, while a homolog of HopPtoS2 is present in *X. c. campestris*. It will be interesting to determine if these proteins have targets and activities in plants that are similar to those of the TTSS-secreted *P. aeruginosa* ADP ribosyltransferases in animals [46–49]. Also, DC3000 encodes two Hops, HopPtoC and HopPtoN, that resemble the YopT-like cysteine protease [7]. Thus, the set of effectors that are common to plant and animal pathogens is growing. Regarding widely conserved Hops, it is noteworthy that *avrPphE* and *hrpW* homologs are also present in *R. solanacearum*, *X. c. campestris*, and *X. a. citri*, and *hopPtoC*, *hopPtoD1*, *hopPtoF*, *hopPtoG*, *hopPtoH* and *hopPtoK* homologs are present in at least one of these pathogens.

Some *P. syringae* effectors are known to possess myristoylation and palmitoylation sites at their amino termini and localize to the plasma membrane inside plant cells [50,51]. Inspection of the predicted amino-terminal ends of the effectors listed in Table 2 reveals that HopPtoF, HopPtoJ and HopPtoS1 possess potential myristoylation and palmitoylation sites. Interestingly, the HopPtoS2 and HopPtoS3 ADP ribosyltransferases lack these sites and could be targeted elsewhere. HopPtoF is noteworthy for an additional reason. This effector is a homolog of AvrPphF ORF2, which has been shown to suppress the defense-associated hypersensitive response that is elicited by another Avr protein [52,53]. Based on this finding and other evidence, it is likely that HopPtoF and many other Hops target plant defense pathways. Indeed, the large inventory of Hops in *P. syringae* could be a response to the plant's ever-evolving innate immune system. For example, pathogens could counter defensive recognition of an important effector by co-injection of a dedicated suppressor effector. The increased Hop inventory should facilitate direct testing for these activities.

Lessons gained from *P. s. tomato* that could be useful for effector mining in other bacteria

Other plant pathogens could have similarly large inventories of TTSS-secreted proteins. Although only

three proteins have been demonstrated to be secreted via a TTSS in *R. solanacearum*, nearly 50 candidates have been identified in the genome based on homology with known effectors in other pathogens or on structural features such as linkage to PIP boxes (highly conserved plant inducible promoters that activate the Hrp regulon in *R. solanacearum* and *Xanthomonas* spp.) and motifs predicting function in eukaryotic cells [54]. Homology with known Hops in other plant pathogens revealed ten candidate TTSS substrates in each of the completed genomes of *X. campestris* pv. *campestris* and *X. axonopodis* pv. *citri* [55]. Presumably, more candidates will be found with the application of additional mining techniques to these genomes, and as each new effector is added to the collective pool, it becomes a new probe for finding additional effectors based on overall similarity, effector motifs or targeting signals.

The recognition of characteristic amino-acid patterns in the amino-terminal regions of TTSS substrates promises to be the most facile method for mining effectors. For example, searching the genome of *R. solanacearum* with the same algorithm used to identify potential TTSS substrates in DC3000 yielded 73 ORFs, including homologs of *P. syringae* Avr proteins AvrPphD, AvrA and AvrPpiC2, and two of the *R. solanacearum* proteins already shown to be TTSS substrates [30]. The TTSS effectors ExoS and ExoT were identified in a similar search of the *P. aeruginosa* genome, as were secreted flagellar proteins in all genomes analyzed [30]. It is noteworthy that all virulence TTSSs are believed to have evolved from an ancestral flagellar secretion system, and all TTSSs share at least nine protein components involved in protein export across the inner membrane [3]. A common gatekeeper controlling entry into the pathway is suggested by this conservation and by observations of heterologous secretion. For example, YplA can be secreted by any of the three TTSSs (flagellar and two related to virulence) in *Yersinia enterocolitica* [56]. We postulate that the pattern of amino acids we observed in the amino-terminal regions of most *P. syringae* Hops represents an ancestral flagellar/TTSS export signal.

Consistent with this hypothesis, YplA and several of the *Yersinia* Yops follow the *P. syringae* pattern. For example, the first 50 amino acids of YopE are 28% Ser and possess the *P. syringae* Hop pattern (as does a secreted YopE mutant carrying a synthetic poly-Ser/Ile in residues 2–8 [24]). However, YopT has an Asp in position 2, YopO has only 6% Ser in its first 50 amino acids, and YopQ has multiple deviations from the pattern and only 8% Ser. Several questions arise. Will mutation analyses demonstrate an export function for the pattern? Can alternative searchable patterns be identified in other effectors? What is the significance (if any) of different pattern classes in TTSS substrates regarding TTSS pathway preference, secretion efficiency and effector delivery hierarchy? And finally, how many TTSS effectors are encoded by the genomes of *Yersinia*, *Salmonella*, *Shigella* and other animal pathogens?

The current effector inventories in these pathogens are based largely on analyses of individual proteins that are secreted in abundance and on a few studies in which sequence data has aided identification of multiple effectors or candidates [57–59]. The application of multiple mining tools is now needed to establish complete effector inventories for all TTSS-dependent pathogens with sequenced genomes. Complete inventories should greatly enhance comparative analyses of the evolution and function of these systems and yield new insights into bacterial pathogenesis in both plant and animal hosts.

Acknowledgements
We thank Philip A. Bronstein, C. Robin Buell, Sheng Yang He, Lisa M. Schechter and Glenn M. Young for helpful suggestions and comments pertaining to this review. Research in the authors' laboratories is supported by the National Science Foundation (NSF) Plant Genome Research Program Cooperative Agreement DBI-0077622, NSF Grant MCB-9982646 (to A.C.), NSF Grant IBN-0096348 (to J.R.A.) and the National Research Initiative Competitive Grants Program of the US Dept of Agriculture Grant 01-35319-10019 (to J.R.A.). The *P. s. tomato* DC3000 genome is being sequenced, with the support of NSF DBI-0077622, by The Institute for Genomic Research under the direction of C. Robin Buell; the imminent completion of a fully annotated sequence promises further insights into the TTSS substrate inventory.

References

1 Galán, J.E. and Collmer, A. (1999) Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* 284, 1322–1328
2 Francis, M.S. *et al.* (2002) Regulation of type III secretion systems. *Curr. Opin. Microbiol.* 5, 166–172
3 Cornelis, G. and van Gijsegem, F. (2000) Assembly and function of type III secretion systems. *Annu. Rev. Microbiol.* 54, 734–774
4 Buttner, D. and Bonas, U. (2002) Port of entry – the type III secretion translocon. *Trends Microbiol.* 10, 186–192
5 Cornelis, G.R. (2002) *Yersinia* type III secretion: send in the effectors. *J. Cell Biol.* 158, 401–408
6 Orth, K. *et al.* (2000) Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease. *Science* 290, 1594–1597
7 Shao, F. *et al.* (2002) A *Yersinia* effector and a *Pseudomonas* avirulence protein define a family of cysteine proteases functioning in bacterial pathogenesis. *Cell* 109, 575–588
8 Mackey, D. *et al.* (2002) RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in *Arabidopsis*. *Cell* 108, 743–754
9 Ham, J.H. *et al.* (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. U. S. A.* 95, 10206–10211
10 van Dijk, K. *et al.* (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 temperature and pH sensitive manner. *J. Bacteriol.* 181, 4790–4797
11 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
12 Staskawicz, B.J. *et al.* (1984) Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proc. Natl. Acad. Sci. U. S. A.* 81, 6024–6028
13 Lorang, J.M. and Keen, N.T. (1995) Characterization of *avr*E from *Pseudomonas syringae* pv. *tomato*: a *hrp*-linked avirulence locus consisting of at least two transcriptional units. *Mol. Plant-Microbe Interact.* 8, 49–57
14 Xiao, Y. *et al.* (1994) Identification of a putative alternate sigma factor and characterization of a multicomponent regulatory cascade controlling the expression of *Pseudomonas syringae* pv. *syringae* Pss61 *hrp* and *hrmA* genes. *J. Bacteriol.* 176, 1025–1036
15 Grimm, C. *et al.* (1995) The *hrpRS* locus of *Pseudomonas syringae* pv. *phaseolicola* constitutes a complex regulatory unit. *Mol. Microbiol.* 15, 155–165
16 Hutcheson, S.W. *et al.* (2001) Enhancer-binding proteins HrpR and HrpS interact to regulate *hrp*-encoded type III protein secretion in *Pseudomonas syringae* strains. *J. Bacteriol.* 183, 5589–5598
17 Hendrickson, E.I. *et al.* (2000) 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
18 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
19 Innes, R.W. *et al.* (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
20 Xiao, Y. and Hutcheson, S.W. (1994) A single promoter sequence recognized by a newly identified alternate sigma factor directs expression of pathogenicity and host range determinants in *Pseudomonas syringae*. *J. Bacteriol.* 176, 3089–3091
21 Alfano, J.R. *et al.* (2000) The *Pseudomonas syringae* Hrp pathogenicity island has a tripartite mosaic structure composed of a cluster of type III secretions genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4856–4861
22 Mudgett, M.B. *et al.* (2000) Molecular signals required for type III secretion and translocation of the *Xanthomonas campestris* AvrBs2 protein to pepper plants. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13324–13329
23 Anderson, D.M. *et al.* (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. U. S. A.* 96, 12839–12843
24 Lloyd, S.A. *et al.* (2001) Targeting exported substrates to the *Yersinia* TTSS: different functions for different signals? *Trends Microbiol.* 9, 367–371
25 Lloyd, S.A. *et al.* (2002) Molecular characterization of type III secretion signals via analysis of synthetic N-terminal amino acid sequences. *Mol. Microbiol.* 43, 51–59
26 Osbourn, A.E. *et al.* (1987) Identification of plant-induced genes of the bacterial pathogen *Xanthomonas campestris* pathovar *campestris* using a promoter-probe plasmid. *EMBO J.* 6, 23–28
27 Boch, J. *et al.* (2002) Identification of *Pseudomonas syringae* pv. *tomato* genes induced during infection of *Arabidopsis thaliana*. *Mol. Microbiol.* 44, 73–88
28 Fouts, D.E. *et al.* (2002) Genome wide identification of *Pseudomonas syringae* pv. *tomato* DC3000 promoters controlled by the HrpL alternative sigma factor. *Proc. Natl. Acad. Sci. U. S. A.* 99, 2275–2280
29 Zwiesler-Vollick, J. *et al.* (2002) Identification of novel *hrp*-regulated genes through functional genomic analysis of the *Pseudomonas syringae* pv. *tomato* DC3000 genome. *Mol. Microbiol.* 45, 1207–1218
30 Petnicki-Ocwieja, T. *et al.* (2002) Genome wide identification of proteins secreted by the Hrp type III protein secretion system of *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. U. S. A.* 99, 7652–7657
31 Guttman, D.S. *et al.* (2002) A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae*. *Science* 295, 1722–1726
32 Roine, E. *et al.* (1997) Hrp pilus: an *hrp*-dependent bacterial surface appendage produced by *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. U. S. A.* 94, 3459–3464
33 Jin, Q. and He, S-Y. (2001) Role of the Hrp pilus in type III protein secretion in *Pseudomonas syringae*. *Science* 294, 2556–2558
34 Li, C-M. *et al.* (2002) The Hrp pilus of *Pseudomonas syringae* elongates from its tip and acts as a conduit for translocation of the effector protein HrpZ. *EMBO J.* 21, 1909–1915
35 Hoyos, M.E. *et al.* (1996) The interaction of $Harpin_{Pss}$, with plant cell walls. *Mol. Plant-Microbe Interact.* 9, 608–616
36 Lee, J. *et al.* (2001) A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene *HIN1* independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. *Plant Cell* 13, 1079–1093
37 Charkowski, A.O. *et al.* (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 to pectate. *J. Bacteriol.* 180, 5211–5217
38 Yuan, J. and He, S.Y. (1996) The *Pseudomonas syringae* Hrp regulation and secretion system controls the production and secretion of multiple extracellular proteins. *J. Bacteriol.* 178, 6399–6402
39 Buttner, D. *et al.* (2002) Functional analysis of HrpF, a putative type III translocon protein from *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.* 184, 2389–2398
40 Hersh, D. *et al.* (1999) The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc. Natl. Acad. Sci. U. S. A.* 96, 2396–2401
41 Casper-Lindley, C. *et al.* (2002) Direct biochemical evidence for type III secretion-dependent translocation of the AvrBs2 effector protein into plant cells. *Proc. Natl. Acad. Sci. U. S. A.* 99, 8336–8341
42 Sory, M-P. and Cornelis, G.R. (1994) Translocation of a hybrid YopE-adenylate cyclase from *Yersinia enterocolitica* into HeLa cells. *Mol. Microbiol.* 14, 583–594
43 Lahaye, T. and Bonas, U. (2001) Molecular secrets of bacterial type III effector proteins. *Trends Plant Sci.* 6, 1360–1385
44 Dangl, J.L. and Jones, J.D.G. (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411, 826–833
45 Staskawicz, B.J. *et al.* (2001) Common and contrasting themes of plant and animal diseases. *Science* 292, 2285–2289
46 Yahr, T.L. *et al.* (1996) Exoenzyme S of *Pseudomonas aeruginosa* is secreted by a type III pathway. *Mol. Microbiol.* 22, 991–1003
47 Krall, R. *et al.* (2002) *In vivo* rho GTPase-activating protein activity of *Pseudomonas aeruginosa* cytotoxin ExoS. *Infect. Immun.* 70, 360–367
48 Kazmierczak, B.I. and Engel, J.N. (2002) *Pseudomonas aeruginosa* ExoT acts *in vivo* as a GTPase-activating protein for RhoA, Rac1, and Cdc42. *Infect. Immun.* 70, 2198–2205
49 Riese, M.J. *et al.* (2001) ADP ribosylation of Arg41 of Rap by ExoS inhibits the ability of Rap to interact with its guanine nucleotide exchange factor, C3G. *Biochemistry* 40, 3289–3294
50 Shan, L. *et al.* (2000) The *Pseudomonas* AvrPto protein is differentially recognized by tomato and tobacco and is localized to the plant plasma membrane. *Plant Cell* 12, 2323–2337
51 Nimchuk, Z. *et al.* (2000) Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell* 101, 353–363
52 Jackson, R.W. *et al.* (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. U. S. A.* 96, 10875–10880
53 Tsiamis, G. *et al.* (2000) Cultivar-specific avirulence and virulence functions assigned to *avrPphF* in *Pseudomonas syringae* pv. *phaseolicola*, the cause of bean halo-blight disease. *EMBO J.* 19, 3204–3214
54 Salanoubat, M. *et al.* (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature* 415, 497–502
55 da Silva, A.C. *et al.* (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* 417, 459–463
56 Young, B.M. and Young, G.M. (2002) YplA is exported by the Ysc, Ysa, and flagellar type III secretion systems of *Yersinia enterocolitica*. *J. Bacteriol.* 184, 1324–1334
57 Worley, M.J. *et al.* (2000) *Salmonella* SsrB activates a global regulon of horizontally acquired genes. *Mol. Microbiol.* 36, 749–761
58 Miao, E.A. and Miller, S.I. (2000) A conserved amino acid sequence direction intracellular type III secretion by *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7539–7544
59 Buchrieser, C. *et al.* (2000) The virulence plasmid pWR100 and the repertoire of the proteins secreted by the type III secretion apparatus of *Shigella flexneri*. *Mol. Microbiol.* 38, 760–771
60 Kobayashi, D.Y. *et al.* (1989) Cloned avirulence genes from the tomato pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. *Proc. Natl. Acad. Sci. U. S. A.* 86, 157–161
61 Vivian, A. and Arnold, D.L. (2000) Bacterial effector genes and their role in host–pathogen interactions. *J. Plant Pathol.* 82, 163–178
62 Kim, J.F. and Alfano, J.R. (2002) Pathogenicity islands and virulence plasmids of bacterial plant pathogens. In *Curr. Top. Microbiol. Immunol.* (Vol. 264) (Hacker, J., ed.), pp. 127–147, Springer–Verlag
63 Noel, L. *et al.* (2001) cDNA-AFLP analysis unravels a genome-wide *hrpG*-regulon in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Microbiol.* 41, 1271–1281
64 Preston, G. *et al.* (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
65 He, S.Y. *et al.* (1993) *Pseudomonas syringae* pv. *syringae* $harpin_{Pss}$: a protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. *Cell* 73, 1255–1266
66 Kim, Y.J. *et al.* (2002) Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell* 109, 589–598
67 Badel, J.L. *et al.* A gene in the *Pseudomonas syringae* pv. *tomato* Hrp pathogenicity island conserved effector locus, *hopPtoA1*, contributes to efficient formation of bacterial colonies *in planta* and is duplicated elsewhere in the genome. *Mol. Plant –Microbe Interact.* (in press)
68 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
69 Pallen, M.J. *et al.* (2001) An abundance of bacterial ADP-ribosyltransferases – implications for the origin of exotoxins and their human homologues. *Trends Microbiol.* 9, 302–307