Subterfuge and Manipulation: Type III Effector Proteins of Phytopathogenic Bacteria

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Abstract

Diverse gram-negative bacteria deliver effector proteins into the cells of their eukaryotic hosts using the type III secretion system. Collectively, these type III effector proteins function to optimize the host cell environment for bacterial growth. Type III effector proteins are essential for the virulence of *Pseudomonas syringae*, *Xanthomonas* spp., *Ralstonia solanacearum* and *Erwinia* species. Type III secretion systems are also found in nonpathogenic pseudomonads and in species of symbiotic nitrogen-fixing *Rhizobium*. We discuss the functions of type III effector proteins of plant-associated bacteria, with an emphasis on pathogens. Plant pathogens tend to carry diverse collections of type III effectors that likely share overlapping functions. Several effectors inhibit host defense responses. The eukaryotic host targets of only a few type III effector proteins are currently known. We also discuss possible mechanisms for diversification of the suite of type III effector proteins carried by a given bacterial strain.

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INTRODUCTION

TTSS: type III secretion system

Type III effector: protein delivered or translocated into host cells via the TTSS

Type III helper proteins: proteins secreted via the TTSS that do not enter the cytoplasm of the host cell

Harpins: proteins secreted in a type III–dependent manner but remain outside the host cell

Hop: Hrp-dependent outer protein

Gram-negative bacterial pathogens use type III secretion systems (TTSS) to deliver type III effector proteins into their eukaryotic host cells. Collectively, these proteins manipulate host cells to optimize the inter- or intracellular environment for pathogen growth and dissemination while subverting host defense responses. Intracellular pathogens of mammals, such as *Salmonella*, *Shigella*, and *Chlamydia*, use type III effectors to enhance or inhibit their phagocytosis by various differentiated host cells and to alter intracellular survival pathways. Individual type III proteins have specific function(s) and act on specific targets to redirect host cell functions (90). For example, the *Salmonella* type III effector SopE acts as a guanine nucleotide exchange factor (GEF) that activates the small GTP binding proteins Cdc42 and Rac1, which in turn regulate actin polymerization and cytoskeleton remodeling (54). As a result, normally nonphagocytic cells engulf invading bacteria into vacuoles in which the bacteria propagate. By contrast, the *Yersinia* type III effector YopT is a cysteine protease that cleaves isoprenyl groups from Rho GTPases, releasing them from their normal plasma membrane location (119). This prevents macrophage-mediated phagocytosis of *Yersinia* that normally clears them from the blood (114). In contrast to these well-documented examples, the functions of most type III effector proteins from plant pathogenic bacteria are not as well characterized. Accumulating evidence suggests that each pathogen strain introduces a collection of type III effectors into host cells to disable host defenses, facilitating pathogen survival and dissemination.

The TTSS is structurally similar to the basal body of the flagellum but has additional components that facilitate host cell contact. The type III pilus spans the bacterial envelope, forms a conduit between the bacteria and the host membrane, and generates a pore to facilitate translocation of type III effector proteins into the host cell. Specialized chaperone proteins often guide incompletely folded type III effector proteins to the cytoplasmic face of the apparatus for ATP-dependent unwinding and entry into the TTSS (3). The regulation of expression, structure, assembly, and trafficking of the TTSS from plant and animal pathogens has been recently reviewed (58) and is not detailed here.

In addition to type III effectors, type III helper proteins needed for proper assembly of the pilus are secreted via the TTSS. Plant pathogens must penetrate the polysaccharide-rich host cell wall before the TTSS can contact the plasma membrane. They secrete helper proteins and harpins (5) into the extracellular milieu. Harpins can induce cell death if applied as purified proteins to host cells. They can form beta-barrel pores in the host membranes in vitro (77, 104) and are potentially involved in promoting access of the type III effectors to the host cytoplasm. Other secreted, but apparently not translocated, proteins include the *Pseudomonas syringae* proteins HrpW, HopAK1 (formerly HopPmaH), and HopAJ1 (HopPmaG) (27). We use the "unified nomenclature" here (80), with old names, when common, in parentheses. Among these, HrpW and HopAK1 have pectate lyase domains. HrpW also has an additional domain

that can independently trigger host cell death in tobacco (28) and HopAJ1 has homology to transglycosylases (53). Hence, this set of helper proteins may have the capacity to locally degrade host cell walls at the tip of the extruding type III pilus, potentially facilitating access to the plasma membrane.

The genes for the TTSS, along with harpins, accessory helper proteins, and specific transcriptional regulatory proteins, are encoded in a cluster of linked operons with the marks of pathogenicity islands, including flanking tRNA genes and unique G+C content (26). Genes for some translocated type III effectors flank the TTSS gene cluster in *P. syringae* and other plant pathogens (4). However, plant pathogen type III effector genes are also scattered throughout the chromosome and on plasmids (7, 27, 61, 113), often flanked by genes encoding their chaperone proteins. Like the TTSS genes, the type III effector genes bear marks of pathogenicity islands and tend to be located in unrelated chromosome positions in different isolates (27, 70).

Delivery of type III effectors into the host cell is essential for the successful life histories of many gram-negative plant-associated bacteria. **Figure 1** presents a stylized plant associated with several plant pathogenic and symbiotic bacteria. *P. syringae* grows as an epiphyte on leaf surfaces (**Figure 1*a***) (15). When these bacteria gain entry via wounds or stomata, they colonize the intercellular space (the apoplast) and cause leaf speck disease (**Figure 1*b***). Unlike several enteric pathogens, plant pathogens do not appear to penetrate vascular tissue. Infection of leaf apoplasts leads to a variety of symptoms, ranging from galls to leaf spots (91). *Xanthomonas campestris* pathovar (pv.) *vesicatoria* also inhabits leaf apoplasts and causes a leaf spotting disease that superficially resembles *P. syringae* (not shown). *Erwinia* species cause fire blight or soft rot disease (98, 125). Soft rot *Erwinia* secrete plant cell wall–degrading enzymes via the type II secretion system and colonize the macerated tissue (**Figure 1*c***). Given their cell wall–degrading prowess, it is unclear why *Erwinia* would require a TTSS for colonization.

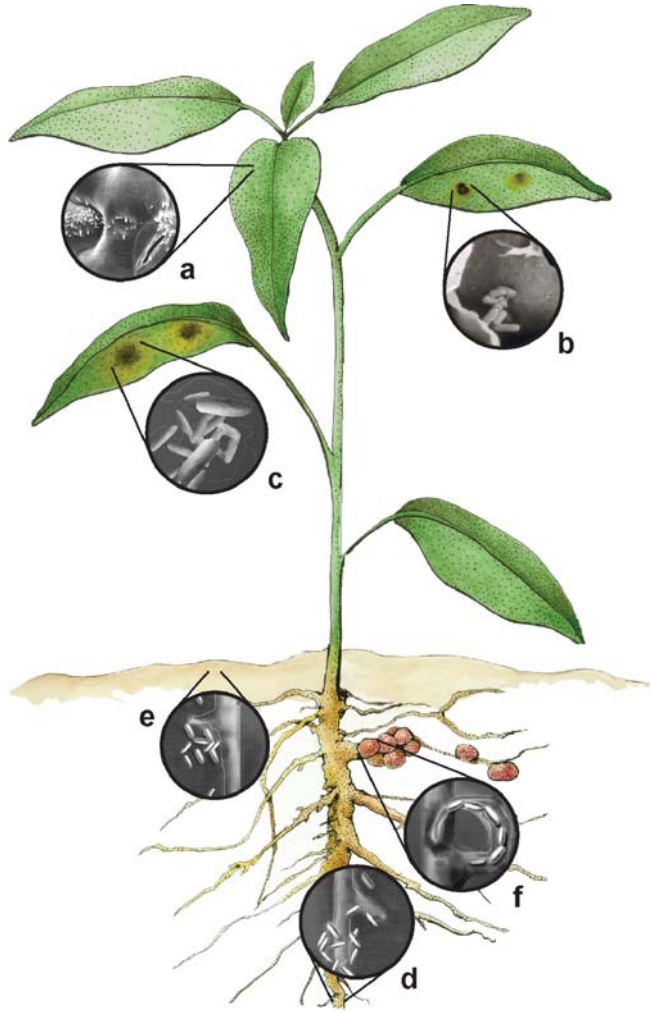

Figure 1

Both pathogenic and symbiotic plant associated bacteria have type III secretion systems. Sites of infection and plant symptoms with pathogenic or symbiotic bacteria are represented on an idealized plant. Pictures are not to scale. (*a*) *Pseudomonas syringae* growing as an epiphyte on a leaf surface next to a stomate (photo is from Gwyn Beattie, Iowa State University). (*b*) Scanning electron microscopy of *P. syringae* in apoplastic space within an infected leaf (reprinted, with permission, from Reference 96). (*c*) *Erwinia carotovora atroseptica* in an infected leaf. (*d*) *Ralstonia solanacearum* associated with a root. (*e*) *Pseudomonas fluorescens* on a root. (*f*) *Rhizobium* spp. in a curling root hair. Nodules are illustrated on the indicated root.

Some strains of biocontrol infect plants through the roots and colonizes the vascular

Pathovar (pv.): the host on which a plant pathogen isolate was collected

PAMPs: pathogen-associated molecular patterns

MAMPs: microbe-associated molecular patterns

Programmed cell death (PCD): cell death induced by a stimulus is defined as programmed if cell signaling is required for cells to die

tissue, causing a wilt disease (49) (**Figure 1*d***). Some strains of biocontrol pseudomonads (*Pseudomonas fluorescens* and *Pseudomonas putida*) that associate with roots protecting plants against fungi and other bacteria also have TTSS genes (103) (**Figure 1*e***). The same is true for some *Rhizobium* species that infect legumes through root hairs (**Figure1*f***). Inside the root, rhizobia establish root nodules where they fix atmospheric nitrogen into ammonia, which can be used as a nitrogen source by the plant (128). Because of their drastically different lifestyles, the nearly ubiquitous presence of TTSS and type III effectors in these species indicates that plant-associated microbes modulate a common mechanism, the host defense response.

The occurrence of TTSS in nonpathogenic pseudomonads and even in species of symbiotic nitrogen-fixing *Rhizobium* is at first glance odd, because we typically associate TTSS with pathogens. A TTSS is found in at least some strains of biocontrol pseudomonads (103, 108), but its necessity for association with plant roots is questionable. Mutations that inactivate the TTSS of *P. fluorescens* strains SBW25 (103) or KD (107) do not interfere with normal root colonization. Other *P. fluorescens* strains that lack a TTSS are nonetheless successful root colonizers (101). A more intriguing role for TTSS in biocontrol pseudomonads was recently suggested by Rezzonico et al. (107), who proposed that biocontrol pseudomonads may actually target plant pathogens using a TTSS. *P. fluorescens* KD carrying a polar mutation in the TTSS-encoding gene cluster was less capable in protecting cucumber from *Pythium ultimum*, a fungus that causes damping-off disease.

In rhizobia, type III effectors that affect host range (87) and bacterial growth in some broad-host-range strains have been identified (9, 13, 120). As with biocontrol pseudomonads, though, many *Rhizobium* strains apparently lack a TTSS but still are able to infect roots, form nodules, and fix nitrogen (86). Type III effector proteins in rhizobia are expressed early in the root hair infection process (73, 128). Potentially, the initial stages of the molecular dialogue of host and bacteria require the latter to suppress host defense until it establishes itself in an "immune privileged" site such as a rhizobial bacteroid.

Diverse plant disease resistance mechanisms can be blocked by type III effector proteins, but type III effector proteins can also trigger the plant immune system. Plant defense mechanisms are based on three general levels of interdiction. The first is structural; most pathogens simply cannot begin their lifestyle on a particular plant surface. The second is guided by the plant's ability to recognize microbial molecular "patterns." These are collectively called pathogen-associated molecular patterns (PAMPs) or, more accurately, microbe-associated molecular patterns (MAMPs) (10). Recognition of these molecules, via specific receptors, induces a large host transcriptional reprogramming that consequently results in biosynthesis of structural barriers and antimicrobials. This response, by definition, is sufficient to stop the growth of nonpathogenic microbes; it is termed the basal defense response (30, 38, 97, 140). Pathogens, by definition, have evolved means to overcome basal defense. This can be deduced from the finding that type III pilus mutants, which no longer grow in planta, actually trigger a more rapid and higher amplitude basal defense response on disease-susceptible hosts compared with wild-type controls (62). Hence, bacterial pathogens use at least part of their type III effector protein collections as virulence factors to dampen or inhibit the plant's basal defense machinery.

However, the type III effectors that allow bacteria to be plant pathogens are a double-edged sword, as plants have evolved a specific branch of their immune system to recognize type III effector action inside the host (30, 38). In fact, plant pathogen type III effector proteins were identified on the basis of their ability to elicit a particular form of host programmed cell death (PCD), called the

hypersensitive response (HR), onto otherwise virulent pathogen strains. HR is determined by the activation of specific host proteins encoded by disease resistance genes. That type III effector proteins triggered HR was also demonstrated, in essence, by Lindgren et al. (81), who showed that the TTSS is essential for initiation of HR on disease-resistant hosts as well as for pathogenicity on disease-susceptible hosts (34).

The majority of plant-specific disease resistance proteins share the structural properties of a nucleotide binding site followed by a variable number of leucine-rich repeats (LRRs). As a class, they are called nucleotide binding (NB)-LRR proteins (30, 38). The *Arabidopsis* genome encodes ~125 different NB-LRR proteins. Functional NB-LRR proteins recognize the presence of a specific bacterial type III effector protein during infection. Because plants are polymorphic for NB-LRR genes and pathogens are polymorphic for the type III effector genes that trigger their action, the interaction between bacterial avirulence (*Avr*) genes and plant *R* genes was called gene-for-gene resistance. While a receptor-ligand model easily explains this aspect of plant immune system genetics, there is little evidence to support it as a generality. By contrast, there is increasing evidence to suggest that the action of type III effector proteins indirectly activates the corresponding NB-LRR protein (38, 127). Examples of this model are detailed below.

Sequencing of complete bacterial genomes, coupled with a variety of effective genetic screens (27, 37, 53, 112, 116), has led to the identification of large numbers of type III effectors in plant pathogens. These screens have been covered in several excellent recent reviews (5, 91, 96). More than 50 potential type III effector families have been identified in *P. syringae* alone (80). In this review, we survey what is known about the virulence functions of type III effector proteins, with an emphasis on those from *P. syringae*. We also examine the distribution of type III effector proteins in several sequenced bacterial genomes currently available in public databases.

The collection of type III effector proteins encoded by different species, or even in different strains within a species, is diverse. Because they are virulence factors, different type III effector proteins may be needed to facilitate distinct infection strategies or to manipulate plant-host-specific targets. Alternatively, at least part of the type III effector diversity may be caused by the need to avoid the plant immune system, as represented by the NB-LRR proteins described above. The successful recognition of a type III effector's action by the corresponding NB-LRR protein has strong negative selective consequences across the microbial population. Hence, it is reasonable to suggest that type III effector diversity and distribution results from a balance between a requirement for multiple type III effector virulence functions to establish a successful infection on a given host species and the need to avoid the devastating consequences that arise if a single type III effector activates a NB-LRR protein in that host.

Hypersensitive response (HR): rapid programmed cell death that occurs at the site of infection if an R protein is activated

Specific disease resistance proteins: proteins activated to induce a strong defense response by the introduction of a specific type III effector protein into host cells

LRR: leucine-rich repeat

NB-LRR protein: nucleotide binding, leucine-rich repeat containing protein

***R* genes:** encode specific disease resistance proteins

FUNCTIONS OF TYPE III EFFECTORS IN VIRULENCE

Subterfuge: Type III Secretion Systems Deliver Proteins into Host Cells that Interfere with Defense Responses

A functional TTSS is essential for *P. syringae*, *R. solanacearum*, *Xanthomonas campestris* pv. *vesicatoria*, and *Erwinia amylovora* to grow in plants (6, 14, 20, 81). The plant responds to TTSS-defective bacteria with a basal defense response involving rapid increases in Ca^{2+} flux and increases in nitric oxide and reactive oxygen species (97). Mitogen-activated protein kinase kinase (MAPKK) pathways are activated (8) and host transcription is dramatically altered (40, 124). Vesicle trafficking is activated, potentially leading to increased secretion of microcidal proteins and compounds and cell wall–strengthening components such

SA: salicylic acid

LPS: lipopolysaccharide

flg22: 22-amino-acid flagellin peptide

Avirulence (Avr) protein: type III effector that activates a specific disease resistance protein

as callose (17, 24, 68). Autofluorescent papilla, rich in callose and phenolics, are produced in cell walls adjacent to infection sites. The plant hormone salicylic acid (SA), which is necessary to stimulate defense responses against biotrophic bacterial pathogens such as *P. syringae*, accumulates (50).

Basal defense responses can also be induced by application of PAMPs such as the purified 22-amino-acid flagellin peptide (flg22) (93, 142), lipopolysaccharide (LPS) derived from pathogen cell walls (68, 137), cold shock protein (48), and the bacterial translation factor EF-Tu (75). Specific receptors for flg22 (FLS2) (51) and EF-Tu have been identified in *Arabidopsis* (141). They are plasma-membrane-bound receptor kinases with extracellular LRRs. Host transcriptional changes induced by activation of the flg22 and EF-Tu receptors are largely overlapping (141). Type III effectors introduced via a functional TTSS appear to block all or parts of the constellation of host responses induced following PAMP perception.

Individual type III effectors interfere with particular defense responses. Deletion of single type III effector genes rarely affects the virulence of the strain in question (56, 130). This suggests that a given pathogen strain expresses functionally overlapping type III effectors that are not homologous, and hence likely represents convergent evolution driven by the need to manipulate a particular host target molecule. Alternatively, a given type III effector may simply not be functional on a given host plant and its gene may be maintained in the strain simply because it is evolutionarily neutral; that gene may still be of potential use on a different host sometime in the strain's future. Type III gene distribution may lead to a rephrasing of the old credo "that which does not kill you will make you stronger."

The lack of easily scored loss-of-function phenotypes for most type III effector mutants has made it difficult to assess their functions in disease. Recent studies have focused on specific aspects of defense to investigate the function of type III effector proteins. These results are summarized in **Table 1**, and some are described below. Much of this work has been recently reviewed (91, 96). In summary, these experiments reveal that individual type III effectors alter some, but not all, aspects of either basal or NB-LRR-mediated specific defense responses. Sequence-unrelated type III effector proteins appear to overlap with respect to which aspects of defense response they can influence.

Some type III effectors block cell wall–mediated defense responses. Callose deposition, a physical block to increased colonization, is stimulated in leaf mesophyll cells when *Arabidopsis* is exposed to *P. syringae* or *X. campestris* pv. *vesicatoria* mutants defective in type III secretion (55, 68) or to flg22 peptide (71). This defense response can be blocked by expression in *trans* of several type III effector proteins in those plant cells (**Table 1**). For example, the avirulence (Avr) protein AvrPto blocks papilla induced via an SA-independent pathway (55). Conditional expression of AvrPto in *Arabidopsis* leads to multiple changes in gene expression, including downregulation of secreted cell wall proteins. By contrast, the type III effector proteins AvrE and HopM1 (HopPtoM) block callose deposited as part of an SA-dependent response pathway. AvrE and HopM1 are functionally redundant but sequence unrelated (41). Deletion of both leads to a significant loss-of-pathogen virulence (at least for the strain tested), but complementation in the bacteria by either AvrE or HopM1 is sufficient to restore virulence of the double mutant to wild-type levels.

Some type III effectors manipulate plant hormone signaling to alter host defense responses. RAP2.6 is an ethylene-responsive transcription factor in *Arabidopsis*. Its expression is induced following infection with virulent *P. syringae* via jasmonic acid (JA) signaling (56). JA is a signaling hormone

Table 1 Type III effectors alter basal defense functions

Defense function	Organism	Type III effector	Previous name	References
Suppress papilla formation	*Pseudomonas syringae*	AvrPto1 AvrE1 HopM1 AvrRpm1 AvrRpt2	AvrPto AvrE HopPtoM AvrRpm1 AvrRpt2	55 41 71
Induce JA-responsive genes	*P. syringae*	AvrB1 AvrRpt2 HopA1 HopD1 HopK1 HopX1 HopAO1	AvrB AvrRpt2 HopPsyA HopPtoD1 HopPtoK AvrPphE HopPtoD2	56
Alter ethylene responses	*P. syringae*	AvrPto1 HopAB2	AvrPto AvrPtoB	33
Suppress cell death induced by specific disease resistance gene	*P. syringae*	AvrRpm1 AvrRpt2 HopAB2 AvrB2 HopF2	AvrRpm1 AvrRpt2 AvrPtoB AvrPphC AvrPphF	110 106 2 126
Suppress cell death induced in response to HopPsyA	*P. syringae*	HopE1 HopF2 HopG1 HopX1 HopAB2 HopAM1	HopE HopF HopG AvrPphE AvrPtoB AvrPpiB	63
Suppress cell death in nonhost plant	*P. syringae*	HopN1 HopAB2 HopAO1	HopPtoN AvrPtoB HopPtoD2	82 2, 60 22, 47
Suppress Flg22-dependent *NHO1* induction	*P. syringae*	AvrPto1 HopC1 HopF2 HopS1 HopT1-1 HopT1-2 HopAA1-1 HopAF1 HopAI-1	AvrPto AvrPpiC2 AvrPphF HolPtoZ HolPtoU1 HolPtoU2 HopPtoA1	78

induced upon wounding or following infection with necrotrophic plant pathogens that rapidly kill host cells (50). JA and SA signaling can be antagonistic (74). Therefore, the activation of JA-induced signaling can repress the SA signaling pathway, which is necessary for effective basal defense against biotrophic pathogens such as *P. syringae* (42, 94, 131). The *P. syringae* phytotoxin coronatine (COR) is a chemical mimic of JA and has the same effect (23). Five of 10 tested type III effector mutants of *P. syringae* pv. *tomato* (*Pto*) DC3000 exhibited significant loss of RAP2.6-induced expression (56) (**Table 1**). At least two other type III effectors, AvrB and AvrRpt2, induced RAP2.6 expression when expressed in a virulent *P. syringae* pathogen infecting susceptible *Arabidopsis* genotypes.

JA: jasmonic acid
COR: coronatine

Cohn & Martin (33) used a cDNA microarray to analyze changes in gene expression in a susceptible tomato line in response to isogenic mutant strains of *Pto* DC3000 lacking either *avrPto*, *hopAB2*, or both. They found that both type III effectors regulate overlapping sets of genes involved in ethylene production and signaling, suggesting that AvrPto and HopAB2 perform similar functions in a compatible host. Ethylene is necessary for chlorotic disease symptom development, but its role in limiting bacterial growth is unclear.

Some type III effectors can interfere with programmed cell death induced by activation of disease resistance proteins. Specific disease resistance protein-mediated transcriptional outputs are similar in quality to responses induced by PAMPS or TTSS-defective bacteria, but are typically more rapid and of greater amplitude (33, 40, 93, 124). HR also nearly always accompanies disease resistance protein action (97). Type III effector proteins can interfere with HR activated following specific recognition of another type III effector protein, hence one can mask the other's presence. For example, HopAB1 (VirPphA) from *P. syrinage* pv. *phaseolicola* (60) can mask the presence of another type III effector that could otherwise trigger the function of a previously unidentified disease resistance protein. *Agrobacterium*-mediated transient expression of another HopAB family member, HopAB2 (AvrPtoB) from *Pto* DC3000 in tomato or tobacco cells, can block cell death induced by the interaction of a number of coexpressed *Avr* and *R* genes. It can also block cell death induced in tobacco or in yeast cells by coexpression of the nonspecific proapoptotic mouse protein Bax (2). Because it interferes with cell death initiated by several different stimuli, HopAB2 is thought to affect a late stage of the cell death pathway. However HopAB2 cannot block HR induced by all disease resistance proteins, and this activity requires its overexpression. For example, *P. syringae* that expresses HopAB2 is recognized by the tomato resistance protein Pto, via the corresponding type III effector protein AvrPto, to induce a HR and an effective defense response.

The *P. syringae* type III effector HopF2 (AvrPphF) can inhibit cell death stimulated in bean cultivars by disease resistance proteins that are activated by other type III effector proteins from the same pathogen. In turn, AvrB2 (AvrPphC) inhibits cell death that is normally activated by HopF2 in bean cultivars that carry the appropriate disease resistance protein (126). Both AvrB2 and HopF2 may inhibit a late component of the cell death response, similar to the HopAB family members. Alternatively, they may interfere with activation of the specific disease resistance protein by the other type III effector. Such interference was initially described because *P. syringae* AvrRpm1 blocks HR elicited by AvrRpt2 and, when using different expression conditions, vice versa (106, 110). This occurs because both type III effectors interact with a common target called RIN4 (69). The same target is necessary for the activation of two different NB-LRR proteins, one activated by AvrRpt2 and the other activated by AvrRpm1, as discussed further below. These results suggest that some type III effector gene combinations may be codistributed in order to negate the undesired activation of specific host disease resistance responses. In other words, some type III effectors may have virulence functions that block the avirulence functions of other type III effectors.

Some type III effectors can interfere with nonhost disease resistance. Several type III effector proteins can block nonhost-induced PCD initiated by expression of HopA1 (HopPsyA) in *Nicotiana tabacum* and *Arabidopsis* (63). With the exception of HopAM1 (AvrPpiB), they also inhibit PCD induced in yeast cells by coexpression of the nonspecific proapoptotic protein from mammalian cells, Bax1. This indicates that these type III effector proteins, like the HopAB family, block a nonspecific step of the PCD pathway. One important caveat from this work is that the

type III effectors ascribed cell death suppression function are normally delivered from a virulent bacterial strain, *Pto* DC3000. This strain is nevertheless capable of triggering specific HR when it carries any of several type III effectors recognized as avirulence proteins by NB-LRR proteins in *Arabidopsis*. Hence, either the cell death suppression activities defined by Jamir et al. (63) act in pathways not involved in normal HR triggered by specific avirulence functions, or their action is diluted below a functional threshold when the signal for HR emanates from an activated NB-LRR protein.

HopAO1 (HopPtoD2) is an important example of another sort of type III effector protein—functional chimeras (22, 47). The HopAO1 C-terminal domain is homologous to a class of tyrosine phosphatases. Its N-terminal domain resembles another *P. syringae* type III effector with no predicted protein function called HopD (HopPtoD). Similar modular type III effectors with C-terminal protein phosphatase domains are found in *Salmonella*, SptP, and *Yersinia*, YopH (66). When expressed in *P. syringae* pv. *phaseolicola*, HopAO1 slows development of a nonhost HR in *Nicotiana benthamiana* (47). Mutations in the tyrosine phosphatase domain restore rapid cell death in this system, indicating that the slowing of cell death depends on the tyrosine kinase activity. Transient expression of an activated MAPKK, $NtMEK^{DD}$, in tobacco also leads to ectopic cell death (135). Coexpression of HopAO1 prevents this, again as long as the HopAO1 tyrosine phosphatase domain is functional. This could indicate that HopPtoD2 blocks HR downstream of NtMEK2 function. HopAO1 does not block HopA1 (HopPsyA)-induced cell death when both are delivered into host cells (63), indicating that a different step in PCD is involved.

Other type III effector proteins can alter parts of the host basal defense transcriptional output. For example, exposure of *Arabidopsis* to flg22 leads to induction of expression of *NHO1* (78), a gene involved in nonhost resistance (65). Loss-of-function *nho1* mutants allow nonhost *P. syringae* strains to grow on *Arabidopsis*, and overexpression of *NHO1* enhances resistance to virulent *Pto* DC3000. Transgenic *Arabidopsis* plants expressing a NHO1-luciferase fusion protein were used to monitor the effects of individual type III effector proteins on flg22-induced NHO1 expression. They transfected protoplasts with 19 type III effectors from *Pto* DC3000 under the control of the strong constitutive 35S viral promoter, exposed the protoplasts to flg22 peptide, and monitored the induction of luciferase. The nine type III effectors listed in **Table 1** can block flg22-dependent activation of the *NHO1* promoter (78).

Nonhost resistance: when all genotypes of a host mount a successful defense response against all genotypes of a pathogen

MAPK: mitogen-activated protein kinase

Manipulation: Type III Effectors Target Host Proteins and Modify their Normal Cellular Function

The enzyme function of a small number of type III effectors has been predicted from homology to known proteins. **Table 2** lists type III effector proteins from plant pathogens with known or predicted enzymatic function. Several types of proteases have been identified. *P. syringae* homologs to the *Yersinia* protease YopT include HopN1 (HopPtoN), HopAR1 (AvrPphB), and HopC1 (HopPtoC) (25, 83, 119). Enzyme targets in plant hosts of the proteases HopAR1 (AvrPphB) and AvrRpt2 have also been identified and their role in plant defense is discussed below.

X. campestris pv. *vesicatoria* (91), *P. syringae* (7), *R. solanacearum* (43) and *E. amylovora* (98) have widely distributed type III effector proteins with homology to the *Yersinia* effector YopJ, which inhibits MAPK and Nfκ-B signaling in pathogen-infected animal cells (99). Various SUMO proteases are found in *X. campestris* pv. *vesicatoria*. The YopJ family member AvrBsT has SUMO protease activity in vitro (100). A second YopJ family member, AvrXv4, can cleave SUMO modifications from multiple substrates in plants (91, 111), but a specific target has not been identified. Although they are members of the same

Table 2 Type III effectors with known or predicted enzyme functions

Function	Organism	Type III effector	Previous name	References
Papain-like cysteine protease, YopT-like	*Pseudomonas syringae*	HopC1 HopN1 HopAR1	HopPtoC HopPtoN AvrPphB	25 82 119, 138
Staphopain cysteine protease	*P. syringae*	AvrRpt2	AvrRpt2	32
C48 family ubiquitin-like SUMO protease	*Xanthomonas campestris*	XopD	XopD	59
YopJ-like SUMO protease	*P. syringae*, *X. campestris*	HopZ2 AvrXv4 AvrBsT AvrRxv	AvrPpiG AvrXv4 AvrBsT AvrRxv	7 111
	Ralstonia solanacearum	PopP1 PopP2	PopP1 PopP2	43, 76
Protein tyrosine phosphatase	*P. syringae*	HopAO1	HopPtoD2	47
Ubiquitin E3 ligase	*P. syringae*	HopAB2 C terminus	AvrPtoB	64
Transcription factor	*Xanthomonas* spp.	AvrBs3/PthA family	AvrBs3/PthA family	123, 134
Glycerophosphoryl diester phosphodiesterase	*X. campestris* pv. *vesicatoria*	AvrBs2	AvrBs2	122
Syringolide synthase	*P. syringae*	AvrD1	AvrD	89

protein family as YopJ, there is no evidence that they affect MAPK signaling in plants. XopD is a highly diverged, c48 family ubiquitin-like SUMO protease, and its activity in plant substrates has also been demonstrated in vitro (59).

HopAB family members (**Tables 1** and **2**) share at least an N-terminal homology of unknown function. The related HopAB1 and HopAB2 subfamilies (exemplified by VirPphA and AvrPtoB, respectively) also share related C-terminal domains. The C terminus of HopAB2 encodes a functional U-box E3 ubiquitin ligase (64). This domain is necessary and sufficient for the cell death inhibition function detailed below. Oddly, some HopAB3 subfamily members, such as *P. syringae* HopPmaL and HopPmaN, contain truncated C termini that lack the E3 ligase domain (79). The function of these truncated family members is unclear. These comparative analyses suggest that bacterial pathogens have genetic mechanisms to mix and match domains that might provide selective broadening of the host target range while conserving a basic function. One can imagine that the HopAB family shares an N-terminal domain to grab a particular host protein(s), and these targets, once bound, might be subjected to different fates depending on the C-terminal domain present in the particular HopAB subfamily in question.

The AvrBs3/PthA family of type III effector proteins is widely distributed in *Xanthomonas* species (26, 67, 136, 139). AvrBs3/PthA family members have features of transcription factors. Their C termini contain an acidic activation domain and three nuclear localization sites. A family member from *Xanthomonas oryzae* pv. *oryzae*, Xa7, binds AT-rich DNA (134), but specific host DNA binding sites have not been identified. The acidic activation domain of Xa10 can activate transcription in yeasts and *Arabidopsis* (139). The family is also defined by a series of 34 base pair repeats encoding the central portion of the protein. The number of repeats can have functional consequences, though it is not understood how these differences are achieved. AvrBs3 interacts with α-importin, as expected if it is transferred to the host nucleus (123). Simultaneous deletion of several *avrBs3/pthA* family members severely reduces virulence of

X. oryzae pv. *oryzae* on rice (136). Infection of pepper leaves with *X. campestris* pv. *vesicatoria* expressing *avrBs3* leads to pustule formation as a disease symptom, and transient expression of AvrBs3 protein in tobacco cells leads to host cell hypertrophy (88). Consistent with their potential function as a transcription factor, many host genes are activated when susceptible pepper is infected with *X. campestris* pv. *vesicatoria* expressing *avrBs3*, including an excess of auxin-inducible genes and expansin-like protein genes.

NB-LRR proteins guard the targets of type III effectors. Type III effector proteins from plant pathogens were initially identified when they were recognized by the products of specific disease resistance genes in specific disease-resistant genotypes of host plant. As outlined above, accumulating evidence indicates that plant disease resistance proteins do not interact directly with their cognate pathogen type III effector in a simple receptor-ligand manner. Instead the plant immune system, via its NB-LRR disease resistance proteins, monitors the action of type III effectors on other plant proteins. These host proteins are most likely type III effector targets that the pathogen manipulates to further its fitness (30, 38). In this model, type III effector proteins (and presumably virulence factors from all pathogen classes) could target a finite number of host protein machines to block basal defense, stimulate pathogen nutrient acquisition, or facilitate pathogen dissemination. NB-LRR proteins, in turn, guard these protein machines and recognize their perturbation by pathogen virulence factors. This, in turn, triggers an amplified and accelerated defense response that is nearly always associated with HR at the site of infection.

HopAR1 targets a kinase, PBS1. One genetic consequence of this model is that a host target of type III effector action could be identified as a protein required for activation of a specific NB-LRR protein. The *P. syringae* type III effector HopAR1 (AvrPphB) is a papain-like cysteine protease in the YopT family (119, 138) (**Table 2**). Its target was identified in a mutation screen for genes required for activation of the *Arabidopsis* RPS5 NB-LRR resistance protein, which recognizes HopAR1. HopAR1 specifically cleaves PBS1 (118), a serine-threonine kinase conserved across plant species (121). If RPS5 is present, cleavage of PBS1 activates a defense response that stops the growth of the infecting pathogen. Following cleavage, PBS1 catalytic residues are still required for RPS5 activation, suggesting that its kinase function is relevant (118). The function of PBS1 in plants that lack RPS5 (and hence are susceptible to bacterial strains expressing HopAR1) is unknown. A *pbs1* mutant is not more resistant or susceptible to virulent pathogens than wild type, which means that PBS1 is not a necessary component of basal plant defense (129).

AvrRpt2, AvrRpm1, and AvrB target RIN4, a repressor of basal defense. If type III effector proteins distributed throughout the pathogen population are functionally redundant with respect to the host proteins they target, one would expect to find some that converge onto a common target(s). Three sequence-unrelated *P. syringae* type III effectors, AvrRpm1, AvrB, and AvrRpt2, do converge onto a conserved, plant-specific *Arabidopsis* protein of unknown function called RIN4. An additional corollary is that any host protein that is independently targeted by pathogen virulence factors might be protected by more than one NB-LRR protein. This is especially true if the mode of action of the type III effectors converging onto that target perturbs its function in different ways. This is also true, since RPM1 and RPS2, two *Arabidopsis* NB-LRR proteins, are activated following different manipulations of RIN4 by the three type III effector proteins listed above.

RPM1 is associated with the plasma membrane (21). It is activated by either AvrB or AvrRpm1, each of which is myristoylated, and possibly palmitoylated, in the host and subsequently also localized to the plasma

membrane (18, 52, 95). RIN4 is also acylated into the plasma membrane (69) and can be coimmunoprecipitated with RPM1 and with either AvrRpm1 or AvrB (85). RIN4 also interacts with both RPM1 and AvrB in yeast two-hybrid experiments (85). When plants are infected with *P. syringae* expressing either AvrB or AvrRpm1, RIN4 becomes hyperphosphorylated (85) (**Figure 2*a***). If RPM1 is present, a disease resistance response is activated. It is still unclear what role RIN4 hyperphosphorylation plays in activation of RPM1.

RPS2 is also associated with the plasma membrane and is activated by AvrRpt2. AvrRpt2 is a staphopain-class cysteine protease (12) (**Table 2**). Like RPM1, RPS2 can coimmunoprecipitate with RIN4 in plants and interact with it in yeast two-hybrid assays (12, 84). When AvrRpt2 is delivered into host cells, it is activated (92) by a host cyclophilin (32) via autocleavage that removes its N-terminal sequences (**Figure 2*b***). The activated protein then cleaves RIN4 at two sequences that are actually homologous to its own autocleavage site (11, 39, 69). Cleavage of RIN4 activates RPS2-mediated disease resistance.

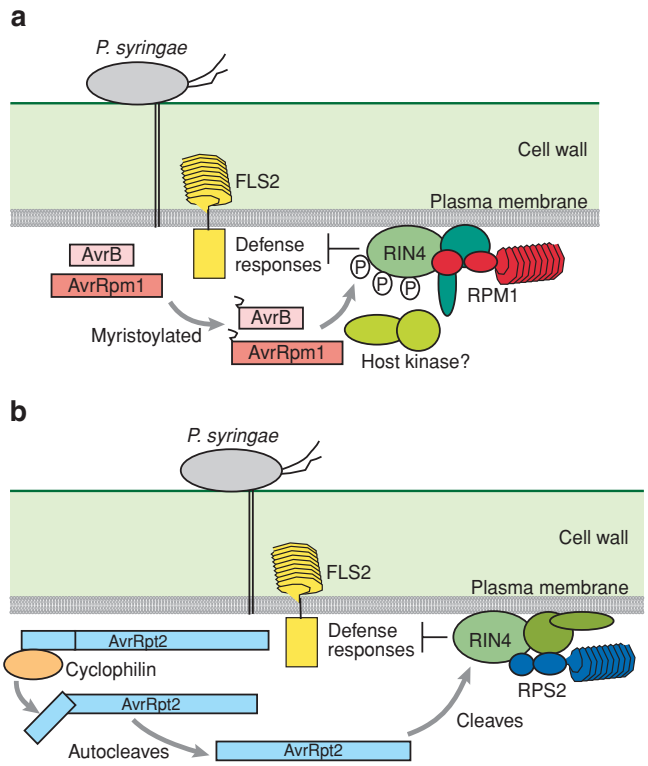

Figure 2

RIN4 is a host target of multiple type III effectors. The activation steps leading to (*a*) hyperphosphorylation as a result of AvrB or AvrRpm1 action, perhaps in association with other host proteins, and (*b*) cleavage of RIN4 by AvrRpt2 are illustrated. RIN4 is assumed to be part of a protein complex at the plant membrane that may include resistance proteins, depending on the host genotype. Other potential virulence targets are not included in the illustration. Objects are not drawn to scale.

RIN4 is a negative regulator of basal defense responses. The observation that three different type III effectors from diverse *P. syringae* strains target RIN4 suggests it has an important role in plant defense. Kim et al. (71) demonstrated that RIN4 is a negative regulator of basal defense mechanisms stimulated by flg22. Overexpression of RIN4 in plants that lack *RPM1* and *RPS2* suppresses several markers of basal defense following either infection with a TTSS-defective *P. syringae* or treatment with flg22 peptide. A *rin4* mutant plant responds to flg22 with enhanced callose induction, and the growth of virulent *Pto* DC3000 is partially inhibited. As a negative regulator of basal defenses, RIN4 is an attractive target for modification by type III effectors. Its hyperphosphorylation by AvrRpm1 or AvrB may stabilize RIN4 in its negative regulatory state. It is not obvious how cleavage of RIN4 by AvrRpt2 can maintain its negative regulatory function, but as an essential part of a protein machine, elimination of RIN4 may prevent subsequent activation of defense components held in that complex. For example, elimination of RIN4 by AvrRpt2 prevents activation of RPM1 by AvrB or AvrRpm1 (84, 110). Alternatively, AvrRpt2 might eliminate RIN4 and other proteins that (*a*) contain the same cleavage site and (*b*) are positive regulators of basal defense. Several such candidates exist (see below).

RIN4 is not the virulence target of AvrRpm1 and AvrRpt2. AvrRpm1 and AvrRpt2 are demonstrably virulence factors

(29, 109). However, their ability to enhance pathogen growth is not lost in a *rin4* mutant, the expectation if RIN4 were the only virulence target of either AvrRpm1 or AvrRpt2 (16). Therefore RIN4 is not required for either of these type III effectors to block basal defenses. RIN4 contains two short domains of roughly 25 amino acids that contain the AvrRpt2 cleavage sites and, in one case, the AvrB binding site, and these are found in a set of additional small *Arabidopsis* proteins that share no other homology (31, 69). It is possible that the primary (virulence) functions of AvrRpm1, AvrB, and AvrRpt2, depend on modification of some, or all, of these proteins. If type III effector proteins generally target multiple host proteins, disease would then be the cumulative result of this multiplicity of manipulations. Disease resistance, though, can be achieved by a particular manipulation activating a single NB-LRR protein. This would be a reasonably efficient way for the plant immune system to achieve "coverage" of the whole set of vulnerable host machines with a limited number of NB-LRR proteins (~125 in the *Arabidopsis* genome).

AvrPto and HopAB2 (AvrPtoB) target the tomato Pto kinase. Both AvrPto and HopAB2 interact physically with the serine-threonine Pto kinase of tomato (2). Although otherwise structurally different, they both contain a short sequence, GINP, that is required for the interaction of AvrPto with Pto in a yeast two-hybrid assay (72, 133). HopAB2 interacts with Pto even if the GINP domain is deleted (2). Both AvrPto and HopAB trigger resistance on tomato plants that express Pto, and this disease resistance response requires the NB-LRR protein Prf (115). According to the guard hypothesis, the NB-LRR protein Prf monitors the interaction of either AvrPto or HopAB2 on Pto to trigger PCD (127). Like RIN4, Pto is not the sole virulence target of AvrPto and HopAB2 because both contribute to virulence on tomato genotypes that lack Pto (79, 117). As summarized in **Table 1**, AvrPto suppresses cell wall–mediated defenses and HopAB2 suppresses PCD. As detailed above, HopAB has a C-terminal domain that suppresses PCD and encodes a U-box class E3 ubiquitin ligase (1, 64). This domain is not necessary for the interaction of HopAB2 with Pto. It is currently unclear how manipulation of Pto by these type III effector proteins activates disease resistance via Prf. There are gain-of-function *Pto* alleles that are active and Prf dependent in the absence of AvrPto (105). Whereas Pto kinase activity is required for AvrPto-dependent Prf activation, it is dispensable for signaling by the constitutively active *Pto* allele. These results suggest that Pto signals the activation of Prf through conformational changes rather than phosphorylation of downstream substrates (132).

How does the interaction of AvrPto and HopAB with Pto modulate basal defense? Recently, AvrPto and HopAB2 were identified in a directed test of known type III effectors that could interfere with flg22 induction of early basal defense responses. Expression of FRK1, an *Arabidopsis* receptor-like kinase, is induced after application of flg22 to plant cells (8). Protoplasts from transgenic *Arabidopsis* carrying a FRK1 promoter-luciferase fusion were transfected with type III effector genes expressed from a constitutive promoter, and the flg22-dependent induction of luciferase expression was monitored. Of 11 type III effectors tested, only AvrPto and HopAB2 blocked FRK1 expression at a step before the initiation of a MAPK cascade (57). AvrPto and HopAB also blocked basal defense activation by other PAMPS (57). One prediction from these results is that AvrPto and HopAB2 interact with an *Arabidopsis* Pto ortholog to dampen early activation steps in PAMP recognition.

Distribution of Type III Effectors

Diverse plant pathogens have unique collections of type III effectors. Plant pathogens with diverse infection strategies, and plant-associated bacteria that can either protect plants from fungal pathogens or live as

intimately associated symbionts, deploy type III effector proteins, as detailed above. We first surveyed the distribution of type III effector protein families across this variety of plant-associated bacterial species. We created a query database of 98 type III effector protein families using TribeMCL with an e-value of 10^{-8} and an inflation value of 1.0 (45). *P. syringae* type III effector protein families were either experimentally confirmed as translocated (27) or identified as type III effector candidates by a variety of computational methods (80) (**Table S1**, follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org/**).

Each type III effector protein sequence was used as a query for a BLASTp search against the genomes of nine fully sequenced plant pathogen genomes (three pathovars of *P. syringae*, *R. solanacearum* GMI1000, two xanthomonads, and three species of *Erwinia*), four soil microbes (three strains of *P. fluorescens* and *P. putida* KT2440), seven mammal pathogens (*Escherichia coli* 0157:H7, two strains each of *Yersinia pestis*, *Salmonella enterica*, and *Shigella flexneri*), and four symbiotic rhizobia (*Sinorhizobium meliloti*, *Rhizobium leguminosarum*, *Mezorhizobium loti*, and *Bradyrhizobium japonicum*), as well as the symbiotic megaplasmid sequence from *Rhizobium* spp. NGR234. BLASTp was performed with an e-value of 10^{-10}. We also included the harpins described above and the ATPase domain of the *Yersinia* TTSS apparatus, YscN, which acts as a positive control for the TTSS in these strains.

Figure 3 presents the results of this survey, focused on the distribution of the type III effector families present in *P. syringae* (27, 80). The results are clustered by bacterial strain. Here, the strains or species with the most similar type III effector protein collections are closest together (right). The phylogeny of these genomes, based solely on the limited number of type III effector protein families displayed here, does not match the

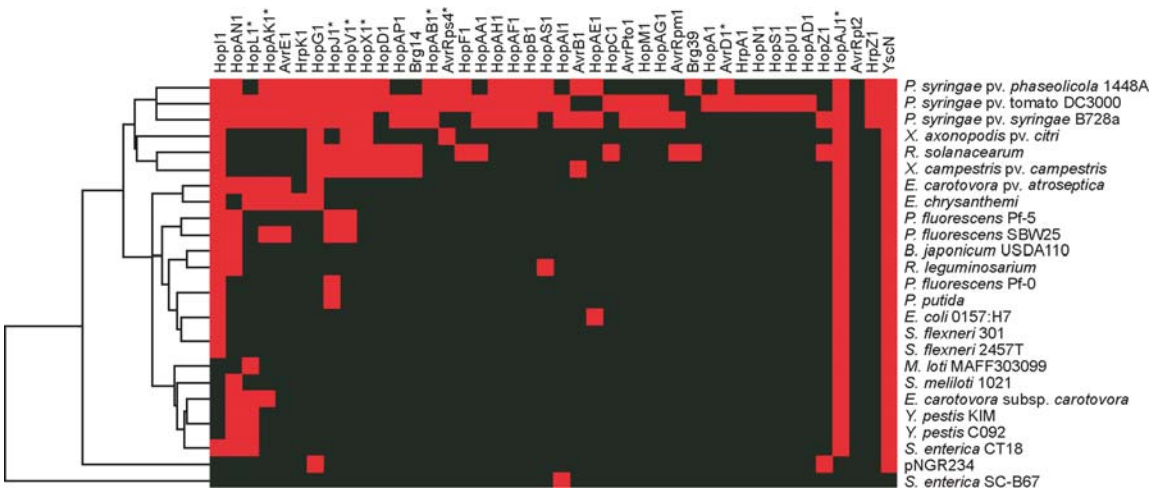

Figure 3

Distribution of effector protein sequences throughout bacterial genomes. BLASTp results are arranged by protein family (see supplemental **Figure S1**) along the top. The results are clustered by the similarity of type III effector protein collections in each bacterial species surveyed. The clusters were created using Cluster 3.0 (**http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/**) based on data by Eisen et al. (44), using a value of 1 for "presence" (*red*) and 0 for "absence" (*black*), and visualized using JavaTreeView (**http://jtreeview.sourceforge.net/**). Asterisk indicates that at least one member of this protein family was not able to be translocated as assayed by delivery of a truncated AvrRpt2 fusion (27).

phylogeny created by MLST analysis using housekeeping protein sequences from AcnA, GltA, GapA, and GyrB protein sequences (46) (data not shown). This strongly suggests that the type III effector suite in any strain is likely to be strongly influenced by horizontal transfer.

It is also evident from **Figure 3** that some type III effector proteins co-occur often. For example, members of the HopI1, HopAN1, and HopL1 families are widely codistributed among both plant and animal pathogens as well as in soil microbes. They are found even in strains of *P. fluorescens* that lack TTSS (Pf-0 and Pf-5) (101). Why are these proteins encoded in strains that lack a TTSS? One possibility is that they are ancient genes whose products can, or did, become substrates for the TTSS in some strains. On the other hand, these genes could be evolutionary remnants in genomes that have lost the TTSS-encoding locus. The functions of HopI1 and HopL1 are unknown. HopAN1 family members have a pectate lyase domain (19, 80). HopL1 is secreted, and conflicting evidence suggests that it may be a conserved helper protein (27, 35, 53) (see **http://www.pseudomonas-syringae.org/**).

The suite of type III effectors is unique to each genome analyzed. The type III effector protein suites from the three sequenced strains of *P. syringae* are closely related, and few of these families are found beyond the pseudomonads. To date, 10 type III effector protein families are limited to *P. syringae*, 4 are only in *Xanthomonas* spp., and 5 are only in *Yersinia* (**Figure 3**; **Figure S1**, follow the Supplemental Material link from the Annual Reviews home page at **http://www.annualreviews.org/**). Among the 42 remaining families with more than one member, there is some evidence of assortment between strains, as well as genetic exchange between species (e.g., *popC*). PopC protein family members are cytoplasmic LRR proteins. Because both plant and animal defense mechanisms involve NB-LRR proteins and other proteins with LRRs (38), PopC proteins may block host defense in diverse hosts.

It is possible that a conserved core set of type III effector proteins is required by all *P. syringae* strains to foster that species' lifestyle on evolutionarily diverse host plants. Their broad distribution among *P. syringae* strains suggests that type III effector proteins have not yet managed to attract the evolutionary attention of the plant's specific immune system. In principle, this could imply that they do not perturb a host protein and are gratuitously delivered to the host. Yet, the wide distribution and significant virulence function for at least some of this class (e.g., the HopM family) argue against this proposition. There is also a set of type III effector proteins that are less well distributed among these three *P. syringae* strains. These might be candidates for overlapping functions; more sequence comparisons and high-level association studies across strains are required to evaluate this proposal.

Some *P. syringae* type III effector protein families are also represented in the genomes of *Ralstonia*, *Xanthomonas*, and *Erwinia*, but each species also has a collection of type III effector families unique to that species (**Figure S1**). This pattern suggests that the majority of the type III effector genes evolved after the divergence of plant pathogenic bacteria and perhaps that type III effector genes are rarely exchanged between species by horizontal transmission.

Although our analyses group proteins by overall homology, type III effectors that share enzymatic active sites, such as YopT family members in *P. syringae* and *Yersinia*, are not sufficiently related over their total lengths to be identified as homologs in our search. Thus, while this analysis is useful for analyzing distribution of related type III effectors, it is not necessarily able to discriminate between proteins that have similar functions at potentially different cellular targets. This caveat begs the development of high-throughput informatics-based pipelines to annotate possible functional

domains present in otherwise divergent protein families.

CONCLUSIONS

Plant pathogens encode larger collections of type III effector proteins than do most animal pathogens, and the collection in each strain analyzed is diverse. We suggest that the balance between the need to have enough type III effectors to ensure virulence on susceptible hosts and the need to avoid recognition by plant disease resistance proteins drives pathogens to diversify their type III effector suites. We have highlighted examples of type III effectors that are required for virulence and individual type III effectors that interfere with diverse aspects of plant basal and specific defense responses. Plant basal defense responses are a complex network that involves the transcriptional reprogramming of over 1000 genes (93, 141). We infer that multiple type III effectors are needed to effectively block various branches of this output network. To this end, plant pathogenic bacteria appear to have evolved the capacity to deliver sequence-unrelated type III effector proteins that function in the same, or related, basal defense response pathways. For example, as detailed above, AvrB1, AvrRpm1, and AvrRpt2 all target RIN4 but thus far only rarely cooccur in *P. syringae* strains. This suggests that convergent evolution is driven by the need to manipulate particular host proteins. In addition, multiple type III effectors in a single strain, *Pto* DC3000, can block papilla formation (AvrPto, AvrE, and HopM1) or PCD (**Table 1**).

What evolutionary pressures could drive these assemblages of type III effector genes? We predict that a collection of type III effectors with overlapping function allows a pathogen population to "lose" one or two effectors, in the face of pressure from the host immune function, without necessarily losing virulence. This loss has been observed in the presence or absence of a particular type III effector virulence gene even within multiple strains of closely related *P. syringae* pathovars (109). Plant breeders adding new specific disease resistance genes to plant varieties often find that virulent "races" of pathogen emerge which have lost, or accrued mutations in, the particular type III effector gene whose product triggered the now-defeated specific disease resistance gene (36).

This pathogen evolution step was recently observed by following the emergence of virulent derivative strains from an avirulent strain of *P. syringae* pv. *phaseolicola* that encodes the type III effector protein HopAR1 (102). After infection of beans that carry the corresponding disease resistance gene, virulent derivatives that had deleted a large genomic pathogenicity island carrying *hopAR1* were recovered. With this kind of strategy, pathogens might run short of virulence factors at some point. New type III effector genes can be acquired by horizontal transmission, reactivation of pseudogenes by excision of a transposable element, or by recombination or transposition-based placement of a type III–regulated promoter upstream of a "hibernating" type III effector gene. The prevalence of type III effector genes in pathogenicity islands or on plasmids (7, 60) and the nonconserved location of type III effectors in different genomes of *P. syringae* (27) indicate that horizontal transmission is active in plant pathogens.

SUMMARY POINTS

1. Type III effector proteins are essential for the successful life histories of many gram-negative bacteria.
2. Diverse plant disease resistance mechanisms can be blocked by type III effector proteins, but they can also trigger the plant immune system.

3. Host defenses are complex, involving activation of MAPK pathways, increases in SA signaling, and secretion of toxins, phenolics, and cell wall–strengthening compounds.
4. Type III effectors target specific host proteins, each of which may play a role in some aspect of host defense. As a consequence, multiple type III effectors are needed to effectively block host defense.
5. Structurally diverse type III effectors can have overlapping functions affecting host defense.
6. Because type III effectors can be horizontally transmitted and deletion can lead to increased host range, the collection of type III effectors in each strain can change over time.
7. The collection of type III effectors in any strain is driven by a need to manipulate certain host proteins but also to avoid specific disease resistance mechanisms.

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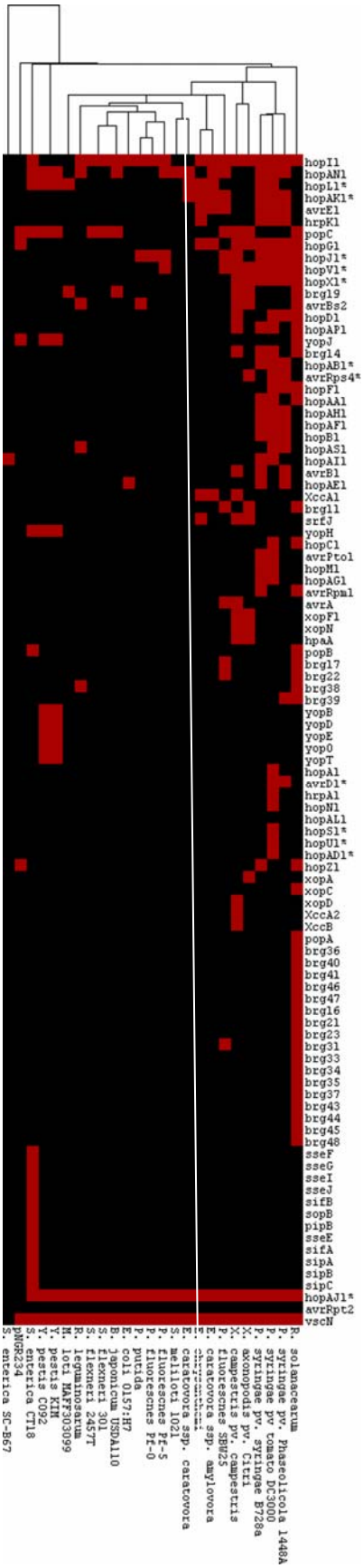
Subterfuge and Manipulation: Type III Effector Proteins of Phytopathogenic Backteria
Grant, Fisher, Chang, Mole, and Dangl

Supplemental figure and table.



Supplemental Figure 1. Distribution of effector protein sequences throughout bacterial genomes. BLASTp results are arranged by protein family along the top. The results are clustered by the similarity of type III effector protein collections in each bacterial species surveyed. The clusters were created using Cluster 3.0(http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/) based on (4) visualized using JavaTreeView. Red indicates presence and black absence.

* indicates that at least one member of this protein family was not able to be translocated as assayed by delivery of a truncated AvrRpt2 fusion (1).

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Grant, Fisher, Chang, Mole, and Dangl

SUPPLEMENTAL TABLE 1 Type III effector protein families

	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
1[a]	HopI1	Psyr4326	*P.syringae*	syringae	B728a	AAY39356
1	HopI1	PSPPHA0012	*P.syringae*	phaseolicola	1448A	YP_272139
1	HopQ1-1	PSPTO0877	*P.syringae*	tomato	DC3000	NP_790716
1	HopQ1-2	PSPTO4732	*P.syringae*	tomato	DC3000	NP_794471
1	Brg20	RSc0245	*R. solanacearum*		GMI1000	NP_518366
1	XopQ		*X.campestris*	vesicatoria	85-10	AAV74206
1	HopI1	HopPmaI	*P.syringae*	maculicola	ES4326	AF458047
1	HopI1	PSPPH4366	*P.syringae*	phaseolicola	1448A	YP_276482
1	HopI1	PSPTO4776	*P.syringae*	tomato	DC3000	NP_794511
2	HopAN1	PSPPH0456	*P.syringae*	phaseolicola	1448A	YP_272759
2	HopAN1	PSPTO5061	*P.syringae*	tomato	DC3000	NP_794793
3	HopL1*	PSPTO2872	*P.syringae*	tomato	DC3000	NP_792673
3	HopL1	Psyr2631	*P.syringae*	syringae	B728a	AAY37670
4	HopAK1	HopPmaH	*P.syringae*	maculicola	ES4326	AF458046
4	HopAK1*	PSPPH1424	*P.syringae*	phaseolicola	1448A	YP_273680
4	HopAK1	Psyr3839	*P.syringae*	syringae	B728a	YP_236907
4	HopAK1*	PSPTO4101	*P.syringae*	tomato	DC3000	PSPTO4101
5	AvrE1		*P.syringae*	maculicola	ES4326	AF458405
5	AvrE1	PSPPH1268	*P.syringae*	phaseolicola	1448A	YP_273527
5	AvrE1	Psyr1188	*P.syringae*	syringae	B728a	YP_234280
5	AvrE1		*P.syringae*	tomato	PT23	U16118
5	AvrE1	PSPTO1377	*P.syringae*	tomato	DC3000	NP_791204
6	HrpK1		*P.syringae*	phaseolicola	1302A race 4	AAA67932
6	HrpK1	PSPPH1295	*P.syringae*	phaseolicola	1448A	YP_273554
6	HrpK1		*P.syringae*	syringae	61	U03855.
6	HrpK1	Psyr1218	*P.syringae*	syringae	B728a	AAP23132
6	HrpK		*P.syringae*	maculicola	ES4326	AAL84241
6	HrpK1	PSPTO1405	*P.syringae*	tomato	DC3000	NP_791232
7	PopC		*R. solanacearum*		GMI1000	NP_522436
7	Brg24	RSp0842	*R. solanacearum*		GMI1000	NP_522403
7	SspH2		*S. enterica*	typhimurium	LT2	NP_461184
7	SspH1		*S. enterica*	typhimurium		AAD40326
7	YopM		*Y.pestis*		KIM	AAC62580
8	HopG1	PSPPH0767	*P.syringae*	phaseolicola	1448A	YP_273053
8	HrpW1		*P.syringae*	maculicola	ES4326	AF458044
8	HopE1	PSPTO4331	*P.syringae*	tomato	DC3000	NP_794087
8	HopG1	PSPTO4727	*P.syringae*	tomato	DC3000	NP_794468
8	PopP1	RSc0826	*R. solanacearum*		GMI1000	CAD14570
8	Brg6	RSc2775	*R. solanacearum*		GMI1000	NP_520896
8	Brg12	RSp0323	*R. solanacearum*		GMI1000	NP_521884
9	HopJ1	HopPmaJ	*P.syringae*	maculicola	ES4326	AF458048
9	HopJ1	PSPPH1068	*P.syringae*	phaseolicola	1448A	YP_273338
9	HopJ1	Psyr1017	*P.syringae*	syringae	B728a	YP_234111
9	HopJ1*	PSPPH0171	*P.syringae*	phaseolicola	1448A	YP_272478
9	HopJ1*	PSPTO1179	*P.syringae*	tomato	DC3000	NP_791011
10	HopV1*	PSPPH2351	*P.syringae*	phaseolicola	1448A	YP_274552
10	HopR1	PSPTO0883	*P.syringae*	tomato	DC3000	NP_790722
10	Brg15	RSp1281	*R. solanacearum*			NP_522840

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
10	HopV1	PSPTO4720	*P.syringae*	tomato	DC3000	NP_794463
11	HopX1	AvrPphE	*P. syringae*	angulata		AAP23110
11	HopX1	AvrPphE	*P. syringae*	delphinii		AAP23116
11	HopX1	AvrPphE	*P. syringae*	glycinia	race 4	AAP23121
11	HopX1	AvrPphE	*P. syringae*	maculicola	ES4326	DQ196428
11	HopX1	AvrPphE	*P. syringae*	maculicola	M6	AF544992
11	HopX1	AvrPphE	*P. syringae*	phaseolicola	1302A race 4	AJ224433
11	HopX1*	PSPPH1296	*P. syringae*	phaseolicola	1448A	YP_273555
11	HopX1	AvrPphE8	*P. syringae*	phaseolicola	B130	AAP23127
11	HopX1	AvrPphE	*P. syringae*	phaseolicola	BK378	AAN85175
11	HopX1	Psyr1220	*P. syringae*	syringae	B728a	AAF71495
11	HopX1	HopPsyE2	*P. syringae*	syringae	W4N 15	AAN85190
11	HopX1	AvrPphE	*P. syringae*	tabaci	ATCC 11528	AAP23130
11	HopX2	HopPmaB	*P. syringae*	maculicola	ES4326	AF458041
11	HopX1	PSPTOA0012	*P. syringae*	tomato	DC3000	AAO53315
11	Brg9	RSc3369	*R.solanacearum*		GMI1000	NP_521488
11	AvrXacE1	AvrXacE1	*X. axonopodis*	citrii	str. 306	NP_640642
11	AvrXacE2	AvrXacE2	*X. axonopodis*	citrii	str. 306	NP_643532
11	XccE1	XccE1	*X. campestris*	campestris	ATCC 33913	NP_636999
12	Brg19	RSc1386	*R. solanacearum*		GMI1000	NP_519507
12	Brg18	RSp0160	*R. solanacearum*		GMI1000	NP_521721
12	XopP		*X.campestris*	vesicatoria	85-10	YP_362967
13	AvrBs2		*X.campestris*	campestris	ATCC33913	NP_635447
14	HopD1		*P.syringae*	glycinea		AJ439731
14	HopD1	AvrPphD	*P.syringae*	phaseolicola	1302A race4	AJ277494
14	HopD1	PSPPHA0010	*P.syringae*	phaseolicola	1448A	YP_272137
14	HopD1		*P.syringae*	savastanoi	ITM317	AJ439730
14	HopD1	PSPTO0876	*P.syringae*	tomato	DC3000	NP_790715
14	HopAO1	PSPTO4722	*P.syringae*	tomato	DC3000	NP_794465
14	Brg8		*R.solanacarum*		GMI1000	NP_521865
14	AvrBs1.1		*X. campestris*	campestris	ATCC 33913	AAM41387
14	XopB		*X. campestris*	vesicatoria	85-10	YP_362312
15	HopAP1	Psyr1890	*P.syringae*	syringae	B728a	YP_234972
15	HopH1		*P.syringae*	pisi	race 1	CAC16702
15	HopH1		*P.syringae*	syringae	B728a	YP_234971
15	HopH1	PSPTO0588	*P.syringae*	tomato	DC3000	NP_790435
15	Brg13	RSc3290	*R. solanacearum*		GMI1000	NP_521409
16	YopJ		*Y.pestis*		KIM	NP_857908
16	HopZ2		*P.syringae*	pisi	race 4A	AJ277495
16	Brg7	RSc0868	*R. solanacearum*		GMI1000	NP_518989
16	XopJ		*X.campestris*	vesicatoria	85-10	CAJ23833
17	Brg14	RSp0572	*R. solanacearum*		GMI1000	NP_522133
18	HopAB1		*P.syringae*	glycinea		AJ439728
18	HopAB1	PSPPHA0127	*P.syringae*	phaseolicola	1448A	YP_272234
18	HopAB1		*P.syringae*	phaseolicola	1449B	AAD47203
18	HopAB1	Psyr4659	*P.syringae*	syringae	B728a	AAY39686
18	HopAB1		*P.syringae*	savastanoi	ETM317	CAD29302
18	HopAB3	HopPmaL	*P.syringae*	maculicola	ES4326	AF458391
18	HopAB3	HolPmaN	*P.syringae*	maculicola	ES4326	AF458050
18	HopAB3*	PSPPH2294	*P.syringae*	phaseolicola	1448A	AAZ35853

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
18	HopAB2	PSPTO3087	*P.syringae*	tomato	DC3000	NP_792881
19	AvrRps4	PSPPHA0087	*P.syringae*	phaseolicola	1448A	YP_272198
19	AvrRps4		*P.syringae*	pisi	151	L43559
19	HopK1	PSPTO0044	*P.syringae*	tomato	DC3000	NP_789904
19	HopAQ1*	PSPTO4703	*P.syringae*	tomato	DC3000	NP_794448
19	XopO		*X.campestris*	vesicatoria	85-10	YP_362786
20	HopF1	AvrPphF	*P.syringae*	phaseolicola	1449A	AF231452
20	HopF2		*P.syringae*	deliphinii	PDDCC529	AAP23118
20	HopF3	PSPPH3498	*P.syringae*	phaseolicola	1448A	YP_275650
20	HopF2	PSPTO0502	*P.syringae*	tomato	DC3000	NP_790351
20	HopAM1	AvrPpiB	*P.syringae*	pisi	870a	X84843
20	HopAM1-1	PSPTO1022	*P.syringae*	tomato	DC3000	NP_790858
20	HopAM1-2	PSPTOA0005	*P.syringae*	tomato	DC3000	NP_808666
21	HopAA1	HopPtoA1	*P.syringae*	maculicola	ES4326	AF458051
21	HopAA1	Psyr1183	*P.syringae*	syringae	B728a	YP_234275
21	HopAA1-1	PSPTO1372	*P.syringae*	tomato	DC3000	NP_791199
21	HopAA1-2	PSPTO4718	*P.syringae*	tomato	DC3000	NP_794461
22	HopAH1	Psyr0779	*P.syringae*	syringae	B728a	DAA00388
22	HopAH2	PSPPH3036	*P.syringae*	phaseolicola	1448A	YP_275213
22	HopAH2	Psyr3123	*P.syringae*	syringae	B728a	YP_236193
22	HopAH1	PSPTO0905	*P.syringae*	tomato	DC3000	NP_790744
23	HopAF1	PSPPH1443	*P.syringae*	phaseolicola	1448A	YP_273699
23	HopAF1	Psyr381	*P.syringae*	syringae	B728a	AAY38843
23	HopAF1	PSPTO1568	*P.syringae*	tomato	DC3000	NP_79139
24	HopB1		*P.syringae*	persicae	5846	AAN85146
24	HopB2		*P.syringae*	syringae	DH015	AAN85183
24	HopB1	PSPTO1406	*P.syringae*	tomato	DC3000	NP_791233
25	HopAS1	PSPPH4736	*P.syringae*	phaseolicola	1448A	YP_276837
25	HopAS1	PSPTO0474	*P.syringae*	tomato	DC3000	NP_790323
26	HopAI1	PSPTO0906	*P.syringae*	tomato	DC3000	NP_790745
27	AvrB1	AvrB	*P.syringae*	glycinea	race 4	M21965
27	AvrB2	AvrC	*P.syringae*	glycinea	race 4	M22219
27	AvrB2		*P.syringae*	phaseolicola	1448A	YP_272229
27	AvrB2	AvrPphC	*P.syringae*	phaseolicola	NPS3121	U10377
27	AvrB3	Psyr1219	*P.syringae*	syringae	B728a	AF232005
27	AvrB3	HopPsyC2	*P.syringae*	syringae	W4N 15	AAN85189
27	XccC		*X.campestris*	campestris	ATCC 33913	ATCC33913
28	HopAE1	PSPPH4326	*P.syringae*	phaseolicola	1448A	YP_276443
28	HopAE1	Psyr4269	*P.syringae*	syringae	B728a	DAA00391
28	HopW1-1	HopPmaA	*P.syringae*	maculicola	ES4326	AF458040
28	HopW1-2		*P.syringae*	maculicola	ES4326	AY603980
29	XccA1		*X.campestris*	campestris	ATCC33913	AAM43445
30	Brg11	RSc1815	*R. solanacearum*		GMI1000	CAD15517
31	SrfJ		*S. enterica*	typhimurium	LT2	NP_463287
32	YopH		*Y. pestis*		KIM	AAC62606
33	HopC1	AvrPpiC	*P.syringae*	pisi	299a race 1	AJ277496
33	HopC1	PSPTO0589	*P.syringae*	tomato	DC3000	NP_790436
33	Brg10	RSc3212	*R. solanacearum*		GMI1000	NP_521333
34	AvrPto1	Psyr4919	*P.syringae*	syringae	B728a	YP_237984
34	AvrPto1		*P.syringae*	tomato	JL1065	L20425

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
34	AvrPto1	PSPTO4001	*P.syringae*	tomato	DC3000	NP_793764
35	HopM1	PSPPH1266	*P.syringae*	phaseolicola	1448A	CP000058
35	HopM1	Psyr1186	*P.syringae*	syringae	B728a	YP_234278
35	HopM1	PSPTO1375	*P.syringae*	tomato	DC3000	NP_791202
36	HopAG1	Psyr0778	*P.syringae*	syringae	B728a	DAA00387
37	AvrRpm1		*P.syringae*	maculicola	M2	X67808
37	AvrRpm1		*P.syringae*	maculicola	M6	AF359557
37	AvrRpm1		*P.syringae*	pisi	race 7	AJ222647
37	AvrRpm1	AvrPpiA	*P.syringae*	pisi	race 2	X67807
37	AvrRpm1	Psyr0738	*P.syringae*	syringae	B728a	AAY35802
38	AvrA		*P.syringae*	glycinea	race 6	M15194
38	AvrBs1		*X.campestris*	campestris	ATCC33913	NP_637464
39	XopF1		*X.campestris*	vesicatoria	85-10	CAJ22045
39	XopF2		*X.campestris*	vesicatoria	85-10	CAJ24621
40	XopN		*X.campestris*	vesicatoria	85-10	CAJ24623
41	hpaA		*X.campestris*	campestris	ATCC33913	NP_636598
41	hpaA		*X.axonopodis*	campestris	ATCC 33913	NP_640755
42	PopB	RSp0876	*R. solanacearum*		GMI1000	NP_522437
42	SpiC	STY1727	*S. enterica*	typhimurium	LT2	NC_003198
43	Brg17	RSc1349	*R. solanacearum*		GMI1000	NP_519470
44	Brg22	RSp0193	*R. solanacearum*		GMI1000	NP_521754
45	Brg38	RSp1031	*R. solanacearum*		GMI1000	NP_522592
46	Brg39	RSp0732	*R. solanacearum*		GMI1000	NP_522293
47	YopB		*Y. pestis*		KIM	NP_857753
48	YopD		*Y. pestis*		KIM	NP_857754
49	YopE		*Y. pestis*		KIM	AAC62587
50	YopO		*Y. pestis*		KIM	AAN37536
51	YopT		*Y. pestis*		KIM	NP_857758
52	HopA1	HopA1	*P.syringae*	syringae	61	AAF71481
52	HopA1	orf2	*P.syringae*	syringae	226	AAP23133
52	HopA2	HopA2	*P.syringae*	atrofaciens	B143	AAP23114
52	HopA2	HopPsyA	*P.syringae*	morsprunorum		AAP23125
52	HopA2	HopPsyB1	*P.syringae*	syringae	464	AY147017
52	HopA2	HopPsyB2	*P.syringae*	syringae	5D4198	AAN85150
52	HopA2	HopPsyB1	*P.syringae*	syringae	B-301D	AAN85156
52	HopA2	HopPsyB1	*P.syringae*	syringae	B5	AAN85164
52	HopA2	HopPsyB1	*P.syringae*	syringae	B6	AAN85168
52	HopA2	HopPsyB1	*P.syringae*	syringae	BK034	AAN85172
52	HopA2	HopPsyB1	*P.syringae*	syringae	PDDCC 3907	AAN85186
52	HopO1-2	PSPTO4594	*P.syringae*	tomato	DC3000	NP_794345
52	HopA1	PSPTO5354	*P.syringae*	tomato	DC3000	NP_795084
52	HopO1-1	PSPTOA0018	*P.syringae*	tomato	DC3000	NP_808677
53	AvrD1	AvrD	*P.syringae*	apii		AF083919
53	AvrD1	AvrD	*P.syringae*	glycinea	race 0	U87225
53	AvrD1	AvrD	*P.syringae*	glycinea	race 1	U87226
53	AvrD1	AvrD	*P.syringae*	glycinea	race 3	U87227
53	AvrD1	AvrD	*P.syringae*	glycinea	race 6	U87227
53	AvrD1	AvrD	*P.syringae*	lacrymans		L11335
53	AvrD1	AvrD	*P.syringae*	lacrymans		L11334
53	AvrD1*	PSPPHA0113	*P.syringae*	phaseolicola	1448A	YP_272223

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
53	AvrD1	AvrD	*P.syringae*	phaseolicola	G50	U87228
53	AvrD1	AvrD	*P.syringae*	phaseolicola	NPS3121	L11336
53	AvrD1	AvrD	*P.syringae*	tomato	PT23	J03681
53	AvrD1	AvrD	*P.syringae*	cilantro		AF083918
53	HopT2	PSPTO4590	*P.syringae*	tomato	DC3000	NP_794341
53	HopT1-2*	PSPTO4593	*P.syringae*	tomato	DC3000	NP_794344
53	HopT1-1*	PSPTOA0019	*P.syringae*	tomato	DC3000	AAO59044
54	HrpA1		*P.syringae*	maculicola	M2	AAQ20005.
54	HopY1	PSPTO0061	*P.syringae*	tomato	DC3000	NP_789920
55	HopN1	PSPTO1370	*P.syringae*	tomato	DC3000	NP_791197
55	HopAL1	HopPmaK	*P.syringae*	maculicola	ES4326	AF458049
56	HopS1*	PSPTO4597	*P.syringae*	tomato	DC3000	NP_794348
56	HopS2*	PSPTO4588	*P.syringae*	tomato	DC3000	NP_794339
57	HopU1*	PSPTO0501	*P.syringae*	tomato	DC3000	NP_790350
58	HopAD1*	PSPTO4691	*P.syringae*	tomato	DC3000	NP_794440
59	HopZ1	HopPmaD	*P.syringae*	maculicola	ES4326	AF458043
59	HopZ1		*P.syringae*	syringae	A2	AAR02168
59	HopZ	Hopz'	*P.syringae*	syringae	B452	AY147021
59	HopZ3	Psyr1224	*P.syringae*	syringae	B728a	AAF71492
60	XopA		*X.campestris*	vesicatoria	85-10	CAJ22071
61	XopC		*X.campestris*	vesicatoria	85-10	CAJ24112
62	XopD		*X.campestris*	vesicatoria	85-10	CAJ22068
63	XccA2		*X.campestris*	campestris	ATCC33913	AAM41674
64	XccB	AvrXccB	*X.campestris*	campestris	ATCC33913	AAM42989
65	PopA	RSp0877	*R. solanacearum*		GMI1000	NP_522438
65	Brg28	RSc1356	*R. solanacearum*		GMI1000	NP_519477
65	Brg29	RSc1357	*R. solanacearum*		GMI1000	NP_519478
65	Brg26	RSc1800	*R. solanacearum*		GMI1000	NP_519921
65	Brg27	RSc1801	*R. solanacearum*		GMI1000	NP_519922
65	Brg25	RSp0672	*R. solanacearum*		GMI1000	NP_522233
66	Brg36	RSc2359	*R. solanacearum*		GMI1000	NP_520480
67	Brg40	RSc3272	*R. solanacearum*		GMI1000	NP_521391
68	Brg41	RSc1475	*R. solanacearum*		GMI1000	NP_519596
69	Brg46	RSc0608	*R. solanacearum*		GMI1000	NP_518729
70	Brg47	RSc3174	*R. solanacearum*		GMI1000	NP_521295
71	Brg16	RSp0213	*R. solanacearum*		GMI1000	NP_518334
72	Brg21	RSp0218	*R. solanacearum*		GMI1000	NP_518339
73	Brg23	RSp0257	*R. solanacearum*		GMI1000	NP_521818
74	Brg31	RSp0099	*R. solanacearum*		GMI1000	NP_521660
74	Brg32	RSp0847	*R. solanacearum*		GMI1000	NP_522408
74	Brg30	RSp1024	*R. solanacearum*		GMI1000	NP_522585
75	Brg33	RSp0845	*R. solanacearum*		GMI1000	NP_522406
76	Brg34	RSp0879	*R. solanacearum*		GMI1000	NP_522440
77	Brg35	RSp0885	*R. solanacearum*		GMI1000	NP_522446
78	Brg37	RSp1022	*R. solanacearum*		GMI1000	NP_522583
79	Brg43	RSp1384	*R. solanacearum*		GMI1000	NP_522943
80	Brg44	RSp1130	*R. solanacearum*		GMI1000	NP_522691
81	Brg45	RSp1460	*R. solanacearum*		GMI1000	NP_523019
82	Brg48	RSp1388	*R. solanacearum*		GMI1000	NP_522947
83	SseF		*S. enterica*	typhimurium	LT2	NP_460369

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
84	SseG		*S. enterica*	typhimurium	LT2	NP_460370
85	SseI		*S. enterica*	typhimurium	LT2	AAL19985
86	SseJ		*S. enterica*	typhimurium	LT2	AE008772
87	SifB		*S. enterica*	typhimurium	LT2	NC_003197
88	SopB		*S. enterica*	typhimurium	LT2	NC_003197
89	PipB		*S. enterica*	typhimurium	LT2	NC_003197
90	SseE		*S. enterica*	typhimurium	LT2	NP_460367
91	SifA		*S. enterica*	typhimurium	LT2	AAL20153
92	SipA	STY3005	*S. enterica*	typhimurium	CT18	NP_457276
93	SipB	STY3008	*S. enterica*	typhimurium	CT18	NP_457279
94	SipC	STY3007	*S. enterica*	typhimurium	CT18	NP_457278
95	HopAJ1	HopPmaG	*P. syringae*	maculicola	ES4326	AF458045
95	HopAJ1*	PSPPH_0763	*P.syringae*	phaseolicola	1448A	AAZ34315.1
95	HopAJ2	PSPPH_4398	*P.syringae*	phaseolicola	1448A	AAZ34636.1
95	HopAJ2	Psyr_4357	*P. syringae*	syringae	B728a	AAY39387.1
95	HopAJ1*	PSPTO0852	*P.syringae*	tomato	DC3000	NP_790692.1
95	HopAJ2	PSPTO4817	*P.syringae*	tomato	DC3000	NP_794551.1
96	AvrRpt2		*P.syringae*	tomato	JL1065	L11355
97	HopAR1	AvrPphB	*P.syringae*	phaseolicola	race3	M86401
98	HrpZ1		*P.syringae*	carambola	HL1	AY372185
98	HrpZ1		*P.syringae*	apata		AF092879
98	HrpZ1		*P.syringae*	glycinia	race 4	L41862
98	HrpZ1		*P.syringae*	maculicola	M2	AY325899
98	HrpZ1		*P.syringae*	phaseolicola	1302A race4	AF268940
98	HrpZ1	PSPPH_1273	*P.syringae*	phaseolicola	1448A	AAZ34500.1
98	HrpZ1		*P.syringae*	pisi	race 1	AB018082
98	HrpZ1		*P.syringae*	pisi	race 2	AB018083
98	HrpZ1		*P.syringae*	pisi	race 3	AB018084
98	HrpZ1		*P.syringae*	pisi	race 4	AB018085
98	HrpZ1		*P.syringae*	syringae	61	L14775
98	HrpZ1	Psyr_1193	*P.syringae*	syringae	B728a	AAY36247.1
98	HrpZ1		*P.syringae*	syringae	LOB201	AB102727
98	HrpZ1		*P.syringae*	syringae	NV	AF031667
98	HrpZ1		*P.syringae*	tabaci	6605	AB049570
98	HrpZ1	PSPTO1382	*P.syringae*	tomato	DC3000	NP_791209
99	YscN		*Y. pestis*		KIM	NP_668159

Supplemental Table 1 Notes:
[a] **Bold font** indicates proteins families represented in Figure 3 of the main text.
[b] Proteins marked (*) are not translocated using full length fusions to truncated AvrRpt2 as an assay (1). Note that other members of some of these families are either not tested, or have been demonstrated to be translocated.
[c]Pathovar

Supplemental Table 1. Analysis: Proteins were identified as type III effectors based on other studies. *P. syringae* were either experimentally confirmed as translocated (1) or identified as type III effector candidates by a variety of computational methods (5). *R. solanacearum* GMI1000 proteins were found based on their *hrp*-dependent transcriptional regulation (2). 41 of these are predicted type III effectors and were included along with known *R. solanacearum* type III effector protein PopC. Other sources for type III effector protein sequences were: *Salmonella enterica* (8); *Yersinia pestis* (7); Xanthomonas spp. (6) and (3).

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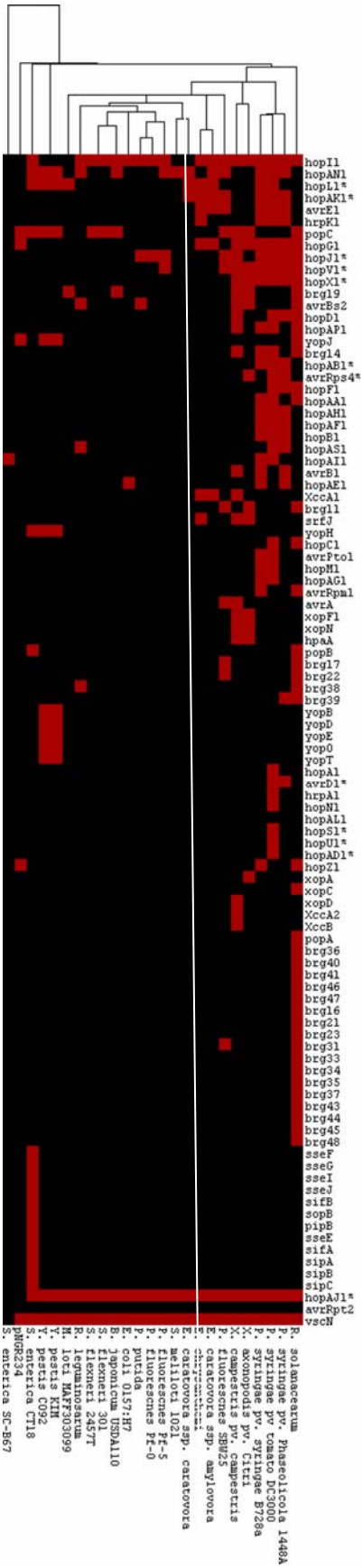
Supplemental figure and table.



Supplemental Figure 1. Distribution of effector protein sequences throughout bacterial genomes. BLASTp results are arranged by protein family along the top. The results are clustered by the similarity of type III effector protein collections in each bacterial species surveyed. The clusters were created using Cluster 3.0(http://bonsai.ims.u-tokyo.ac.jp/~mdehoon/software/cluster/) based on (4) visualized using JavaTreeView. Red indicates presence and black absence.

* indicates that at least one member of this protein family was not able to be translocated as assayed by delivery of a truncated AvrRpt2 fusion (1).

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SUPPLEMENTAL TABLE 1 Type III effector protein families

	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
1[a]	HopI1	Psyr4326	*P.syringae*	syringae	B728a	AAY39356
1	HopI1	PSPPHA0012	*P.syringae*	phaseolicola	1448A	YP_272139
1	HopQ1-1	PSPTO0877	*P.syringae*	tomato	DC3000	NP_790716
1	HopQ1-2	PSPTO4732	*P.syringae*	tomato	DC3000	NP_794471
1	Brg20	RSc0245	*R. solanacearum*		GMI1000	NP_518366
1	XopQ		*X.campestris*	vesicatoria	85-10	AAV74206
1	HopI1	HopPmaI	*P.syringae*	maculicola	ES4326	AF458047
1	HopI1	PSPPH4366	*P.syringae*	phaseolicola	1448A	YP_276482
1	HopI1	PSPTO4776	*P.syringae*	tomato	DC3000	NP_794511
2	HopAN1	PSPPH0456	*P.syringae*	phaseolicola	1448A	YP_272759
2	HopAN1	PSPTO5061	*P.syringae*	tomato	DC3000	NP_794793
3	HopL1*	PSPTO2872	*P.syringae*	tomato	DC3000	NP_792673
3	HopL1	Psyr2631	*P.syringae*	syringae	B728a	AAY37670
4	HopAK1	HopPmaH	*P.syringae*	maculicola	ES4326	AF458046
4	HopAK1*	PSPPH1424	*P.syringae*	phaseolicola	1448A	YP_273680
4	HopAK1	Psyr3839	*P.syringae*	syringae	B728a	YP_236907
4	HopAK1*	PSPTO4101	*P.syringae*	tomato	DC3000	PSPTO4101
5	AvrE1		*P.syringae*	maculicola	ES4326	AF458405
5	AvrE1	PSPPH1268	*P.syringae*	phaseolicola	1448A	YP_273527
5	AvrE1	Psyr1188	*P.syringae*	syringae	B728a	YP_234280
5	AvrE1		*P.syringae*	tomato	PT23	U16118
5	AvrE1	PSPTO1377	*P.syringae*	tomato	DC3000	NP_791204
6	HrpK1		*P.syringae*	phaseolicola	1302A race 4	AAA67932
6	HrpK1	PSPPH1295	*P.syringae*	phaseolicola	1448A	YP_273554
6	HrpK1		*P.syringae*	syringae	61	U03855.
6	HrpK1	Psyr1218	*P.syringae*	syringae	B728a	AAP23132
6	HrpK		*P.syringae*	maculicola	ES4326	AAL84241
6	HrpK1	PSPTO1405	*P.syringae*	tomato	DC3000	NP_791232
7	PopC		*R. solanacearum*		GMI1000	NP_522436
7	Brg24	RSp0842	*R. solanacearum*		GMI1000	NP_522403
7	SspH2		*S. enterica*	typhimurium	LT2	NP_461184
7	SspH1		*S. enterica*	typhimurium		AAD40326
7	YopM		*Y.pestis*		KIM	AAC62580
8	HopG1	PSPPH0767	*P.syringae*	phaseolicola	1448A	YP_273053
8	HrpW1		*P.syringae*	maculicola	ES4326	AF458044
8	HopE1	PSPTO4331	*P.syringae*	tomato	DC3000	NP_794087
8	HopG1	PSPTO4727	*P.syringae*	tomato	DC3000	NP_794468
8	PopP1	RSc0826	*R. solanacearum*		GMI1000	CAD14570
8	Brg6	RSc2775	*R. solanacearum*		GMI1000	NP_520896
8	Brg12	RSp0323	*R. solanacearum*		GMI1000	NP_521884
9	HopJ1	HopPmaJ	*P.syringae*	maculicola	ES4326	AF458048
9	HopJ1	PSPPH1068	*P.syringae*	phaseolicola	1448A	YP_273338
9	HopJ1	Psyr1017	*P.syringae*	syringae	B728a	YP_234111
9	HopJ1*	PSPPH0171	*P.syringae*	phaseolicola	1448A	YP_272478
9	HopJ1*	PSPTO1179	*P.syringae*	tomato	DC3000	NP_791011
10	HopV1*	PSPPH2351	*P.syringae*	phaseolicola	1448A	YP_274552
10	HopR1	PSPTO0883	*P.syringae*	tomato	DC3000	NP_790722
10	Brg15	RSp1281	*R. solanacearum*			NP_522840

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
10	HopV1	PSPTO4720	*P.syringae*	tomato	DC3000	NP_794463
11	HopX1	AvrPphE	*P. syringae*	angulata		AAP23110
11	HopX1	AvrPphE	*P. syringae*	delphinii		AAP23116
11	HopX1	AvrPphE	*P. syringae*	glycinia	race 4	AAP23121
11	HopX1	AvrPphE	*P. syringae*	maculicola	ES4326	DQ196428
11	HopX1	AvrPphE	*P. syringae*	maculicola	M6	AF544992
11	HopX1	AvrPphE	*P. syringae*	phaseolicola	1302A race 4	AJ224433
11	HopX1*	PSPPH1296	*P. syringae*	phaseolicola	1448A	YP_273555
11	HopX1	AvrPphE8	*P. syringae*	phaseolicola	B130	AAP23127
11	HopX1	AvrPphE	*P. syringae*	phaseolicola	BK378	AAN85175
11	HopX1	Psyr1220	*P. syringae*	syringae	B728a	AAF71495
11	HopX1	HopPsyE2	*P. syringae*	syringae	W4N 15	AAN85190
11	HopX1	AvrPphE	*P. syringae*	tabaci	ATCC 11528	AAP23130
11	HopX2	HopPmaB	*P. syringae*	maculicola	ES4326	AF458041
11	HopX1	PSPTOA0012	*P. syringae*	tomato	DC3000	AAO53315
11	Brg9	RSc3369	*R.solanacearum*		GMI1000	NP_521488
11	AvrXacE1	AvrXacE1	*X. axonopodis*	citrii	str. 306	NP_640642
11	AvrXacE2	AvrXacE2	*X. axonopodis*	citrii	str. 306	NP_643532
11	XccE1	XccE1	*X. campestris*	campestris	ATCC 33913	NP_636999
12	Brg19	RSc1386	*R. solanacearum*		GMI1000	NP_519507
12	Brg18	RSp0160	*R. solanacearum*		GMI1000	NP_521721
12	XopP		*X.campestris*	vesicatoria	85-10	YP_362967
13	AvrBs2		*X.campestris*	campestris	ATCC33913	NP_635447
14	HopD1		*P.syringae*	glycinea		AJ439731
14	HopD1	AvrPphD	*P.syringae*	phaseolicola	1302A race4	AJ277494
14	HopD1	PSPPHA0010	*P.syringae*	phaseolicola	1448A	YP_272137
14	HopD1		*P.syringae*	savastanoi	ITM317	AJ439730
14	HopD1	PSPTO0876	*P.syringae*	tomato	DC3000	NP_790715
14	HopAO1	PSPTO4722	*P.syringae*	tomato	DC3000	NP_794465
14	Brg8		*R.solanacarum*		GMI1000	NP_521865
14	AvrBs1.1		*X. campestris*	campestris	ATCC 33913	AAM41387
14	XopB		*X. campestris*	vesicatoria	85-10	YP_362312
15	HopAP1	Psyr1890	*P.syringae*	syringae	B728a	YP_234972
15	HopH1		*P.syringae*	pisi	race 1	CAC16702
15	HopH1		*P.syringae*	syringae	B728a	YP_234971
15	HopH1	PSPTO0588	*P.syringae*	tomato	DC3000	NP_790435
15	Brg13	RSc3290	*R. solanacearum*		GMI1000	NP_521409
16	YopJ		*Y.pestis*		KIM	NP_857908
16	HopZ2		*P.syringae*	pisi	race 4A	AJ277495
16	Brg7	RSc0868	*R. solanacearum*		GMI1000	NP_518989
16	XopJ		*X.campestris*	vesicatoria	85-10	CAJ23833
17	Brg14	RSp0572	*R. solanacearum*		GMI1000	NP_522133
18	HopAB1		*P.syringae*	glycinea		AJ439728
18	HopAB1	PSPPHA0127	*P.syringae*	phaseolicola	1448A	YP_272234
18	HopAB1		*P.syringae*	phaseolicola	1449B	AAD47203
18	HopAB1	Psyr4659	*P.syringae*	syringae	B728a	AAY39686
18	HopAB1		*P.syringae*	savastanoi	ETM317	CAD29302
18	HopAB3	HopPmaL	*P.syringae*	maculicola	ES4326	AF458391
18	HopAB3	HolPmaN	*P.syringae*	maculicola	ES4326	AF458050
18	HopAB3*	PSPPH2294	*P.syringae*	phaseolicola	1448A	AAZ35853

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
18	HopAB2	PSPTO3087	*P.syringae*	tomato	DC3000	NP_792881
19	AvrRps4	PSPPHA0087	*P.syringae*	phaseolicola	1448A	YP_272198
19	AvrRps4		*P.syringae*	pisi	151	L43559
19	HopK1	PSPTO0044	*P.syringae*	tomato	DC3000	NP_789904
19	HopAQ1*	PSPTO4703	*P.syringae*	tomato	DC3000	NP_794448
19	XopO		*X.campestris*	vesicatoria	85-10	YP_362786
20	HopF1	AvrPphF	*P.syringae*	phaseolicola	1449A	AF231452
20	HopF2		*P.syringae*	deliphinii	PDDCC529	AAP23118
20	HopF3	PSPPH3498	*P.syringae*	phaseolicola	1448A	YP_275650
20	HopF2	PSPTO0502	*P.syringae*	tomato	DC3000	NP_790351
20	HopAM1	AvrPpiB	*P.syringae*	pisi	870a	X84843
20	HopAM1-1	PSPTO1022	*P.syringae*	tomato	DC3000	NP_790858
20	HopAM1-2	PSPTOA0005	*P.syringae*	tomato	DC3000	NP_808666
21	HopAA1	HopPtoA1	*P.syringae*	maculicola	ES4326	AF458051
21	HopAA1	Psyr1183	*P.syringae*	syringae	B728a	YP_234275
21	HopAA1-1	PSPTO1372	*P.syringae*	tomato	DC3000	NP_791199
21	HopAA1-2	PSPTO4718	*P.syringae*	tomato	DC3000	NP_794461
22	HopAH1	Psyr0779	*P.syringae*	syringae	B728a	DAA00388
22	HopAH2	PSPPH3036	*P.syringae*	phaseolicola	1448A	YP_275213
22	HopAH2	Psyr3123	*P.syringae*	syringae	B728a	YP_236193
22	HopAH1	PSPTO0905	*P.syringae*	tomato	DC3000	NP_790744
23	HopAF1	PSPPH1443	*P.syringae*	phaseolicola	1448A	YP_273699
23	HopAF1	Psyr381	*P.syringae*	syringae	B728a	AAY38843
23	HopAF1	PSPTO1568	*P.syringae*	tomato	DC3000	NP_79139
24	HopB1		*P.syringae*	persicae	5846	AAN85146
24	HopB2		*P.syringae*	syringae	DH015	AAN85183
24	HopB1	PSPTO1406	*P.syringae*	tomato	DC3000	NP_791233
25	HopAS1	PSPPH4736	*P.syringae*	phaseolicola	1448A	YP_276837
25	HopAS1	PSPTO0474	*P.syringae*	tomato	DC3000	NP_790323
26	HopAI1	PSPTO0906	*P.syringae*	tomato	DC3000	NP_790745
27	AvrB1	AvrB	*P.syringae*	glycinea	race 4	M21965
27	AvrB2	AvrC	*P.syringae*	glycinea	race 4	M22219
27	AvrB2		*P.syringae*	phaseolicola	1448A	YP_272229
27	AvrB2	AvrPphC	*P.syringae*	phaseolicola	NPS3121	U10377
27	AvrB3	Psyr1219	*P.syringae*	syringae	B728a	AF232005
27	AvrB3	HopPsyC2	*P.syringae*	syringae	W4N 15	AAN85189
27	XccC		*X.campestris*	campestris	ATCC 33913	ATCC33913
28	HopAE1	PSPPH4326	*P.syringae*	phaseolicola	1448A	YP_276443
28	HopAE1	Psyr4269	*P.syringae*	syringae	B728a	DAA00391
28	HopW1-1	HopPmaA	*P.syringae*	maculicola	ES4326	AF458040
28	HopW1-2		*P.syringae*	maculicola	ES4326	AY603980
29	XccA1		*X.campestris*	campestris	ATCC33913	AAM43445
30	Brg11	RSc1815	*R. solanacearum*		GMI1000	CAD15517
31	SrfJ		*S. enterica*	typhimurium	LT2	NP_463287
32	YopH		*Y. pestis*		KIM	AAC62606
33	HopC1	AvrPpiC	*P.syringae*	pisi	299a race 1	AJ277496
33	HopC1	PSPTO0589	*P.syringae*	tomato	DC3000	NP_790436
33	Brg10	RSc3212	*R. solanacearum*		GMI1000	NP_521333
34	AvrPto1	Psyr4919	*P.syringae*	syringae	B728a	YP_237984
34	AvrPto1		*P.syringae*	tomato	JL1065	L20425

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34	AvrPto1	PSPTO4001	*P.syringae*	tomato	DC3000	NP_793764
35	HopM1	PSPPH1266	*P.syringae*	phaseolicola	1448A	CP000058
35	HopM1	Psyr1186	*P.syringae*	syringae	B728a	YP_234278
35	HopM1	PSPTO1375	*P.syringae*	tomato	DC3000	NP_791202
36	HopAG1	Psyr0778	*P.syringae*	syringae	B728a	DAA00387
37	AvrRpm1		*P.syringae*	maculicola	M2	X67808
37	AvrRpm1		*P.syringae*	maculicola	M6	AF359557
37	AvrRpm1		*P.syringae*	pisi	race 7	AJ222647
37	AvrRpm1	AvrPpiA	*P.syringae*	pisi	race 2	X67807
37	AvrRpm1	Psyr0738	*P.syringae*	syringae	B728a	AAY35802
38	AvrA		*P.syringae*	glycinea	race 6	M15194
38	AvrBs1		*X.campestris*	campestris	ATCC33913	NP_637464
39	XopF1		*X.campestris*	vesicatoria	85-10	CAJ22045
39	XopF2		*X.campestris*	vesicatoria	85-10	CAJ24621
40	XopN		*X.campestris*	vesicatoria	85-10	CAJ24623
41	hpaA		*X.campestris*	campestris	ATCC33913	NP_636598
41	hpaA		*X.axonopodis*	campestris	ATCC 33913	NP_640755
42	PopB	RSp0876	*R. solanacearum*		GMI1000	NP_522437
42	SpiC	STY1727	*S. enterica*	typhimurium	LT2	NC_003198
43	Brg17	RSc1349	*R. solanacearum*		GMI1000	NP_519470
44	Brg22	RSp0193	*R. solanacearum*		GMI1000	NP_521754
45	Brg38	RSp1031	*R. solanacearum*		GMI1000	NP_522592
46	Brg39	RSp0732	*R. solanacearum*		GMI1000	NP_522293
47	YopB		*Y. pestis*		KIM	NP_857753
48	YopD		*Y. pestis*		KIM	NP_857754
49	YopE		*Y. pestis*		KIM	AAC62587
50	YopO		*Y. pestis*		KIM	AAN37536
51	YopT		*Y. pestis*		KIM	NP_857758
52	HopA1	HopA1	*P.syringae*	syringae	61	AAF71481
52	HopA1	orf2	*P.syringae*	syringae	226	AAP23133
52	HopA2	HopA2	*P.syringae*	atrofaciens	B143	AAP23114
52	HopA2	HopPsyA	*P.syringae*	morsprunorum		AAP23125
52	HopA2	HopPsyB1	*P.syringae*	syringae	464	AY147017
52	HopA2	HopPsyB2	*P.syringae*	syringae	5D4198	AAN85150
52	HopA2	HopPsyB1	*P.syringae*	syringae	B-301D	AAN85156
52	HopA2	HopPsyB1	*P.syringae*	syringae	B5	AAN85164
52	HopA2	HopPsyB1	*P.syringae*	syringae	B6	AAN85168
52	HopA2	HopPsyB1	*P.syringae*	syringae	BK034	AAN85172
52	HopA2	HopPsyB1	*P.syringae*	syringae	PDDCC 3907	AAN85186
52	HopO1-2	PSPTO4594	*P.syringae*	tomato	DC3000	NP_794345
52	HopA1	PSPTO5354	*P.syringae*	tomato	DC3000	NP_795084
52	HopO1-1	PSPTOA0018	*P.syringae*	tomato	DC3000	NP_808677
53	AvrD1	AvrD	*P.syringae*	apii		AF083919
53	AvrD1	AvrD	*P.syringae*	glycinea	race 0	U87225
53	AvrD1	AvrD	*P.syringae*	glycinea	race 1	U87226
53	AvrD1	AvrD	*P.syringae*	glycinea	race 3	U87227
53	AvrD1	AvrD	*P.syringae*	glycinea	race 6	U87227
53	AvrD1	AvrD	*P.syringae*	lacrymans		L11335
53	AvrD1	AvrD	*P.syringae*	lacrymans		L11334
53	AvrD1*	PSPPHA0113	*P.syringae*	phaseolicola	1448A	YP_272223

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
53	AvrD1	AvrD	*P.syringae*	phaseolicola	G50	U87228
53	AvrD1	AvrD	*P.syringae*	phaseolicola	NPS3121	L11336
53	AvrD1	AvrD	*P.syringae*	tomato	PT23	J03681
53	AvrD1	AvrD	*P.syringae*	cilantro		AF083918
53	HopT2	PSPTO4590	*P.syringae*	tomato	DC3000	NP_794341
53	HopT1-2*	PSPTO4593	*P.syringae*	tomato	DC3000	NP_794344
53	HopT1-1*	PSPTOA0019	*P.syringae*	tomato	DC3000	AAO59044
54	HrpA1		*P.syringae*	maculicola	M2	AAQ20005.
54	HopY1	PSPTO0061	*P.syringae*	tomato	DC3000	NP_789920
55	HopN1	PSPTO1370	*P.syringae*	tomato	DC3000	NP_791197
55	HopAL1	HopPmaK	*P.syringae*	maculicola	ES4326	AF458049
56	HopS1*	PSPTO4597	*P.syringae*	tomato	DC3000	NP_794348
56	HopS2*	PSPTO4588	*P.syringae*	tomato	DC3000	NP_794339
57	HopU1*	PSPTO0501	*P.syringae*	tomato	DC3000	NP_790350
58	HopAD1*	PSPTO4691	*P.syringae*	tomato	DC3000	NP_794440
59	HopZ1	HopPmaD	*P.syringae*	maculicola	ES4326	AF458043
59	HopZ1		*P.syringae*	syringae	A2	AAR02168
59	HopZ	Hopz'	*P.syringae*	syringae	B452	AY147021
59	HopZ3	Psyr1224	*P.syringae*	syringae	B728a	AAF71492
60	XopA		*X.campestris*	vesicatoria	85-10	CAJ22071
61	XopC		*X.campestris*	vesicatoria	85-10	CAJ24112
62	XopD		*X.campestris*	vesicatoria	85-10	CAJ22068
63	XccA2		*X.campestris*	campestris	ATCC33913	AAM41674
64	XccB	AvrXccB	*X.campestris*	campestris	ATCC33913	AAM42989
65	PopA	RSp0877	*R. solanacearum*		GMI1000	NP_522438
65	Brg28	RSc1356	*R. solanacearum*		GMI1000	NP_519477
65	Brg29	RSc1357	*R. solanacearum*		GMI1000	NP_519478
65	Brg26	RSc1800	*R. solanacearum*		GMI1000	NP_519921
65	Brg27	RSc1801	*R. solanacearum*		GMI1000	NP_519922
65	Brg25	RSp0672	*R. solanacearum*		GMI1000	NP_522233
66	Brg36	RSc2359	*R. solanacearum*		GMI1000	NP_520480
67	Brg40	RSc3272	*R. solanacearum*		GMI1000	NP_521391
68	Brg41	RSc1475	*R. solanacearum*		GMI1000	NP_519596
69	Brg46	RSc0608	*R. solanacearum*		GMI1000	NP_518729
70	Brg47	RSc3174	*R. solanacearum*		GMI1000	NP_521295
71	Brg16	RSp0213	*R. solanacearum*		GMI1000	NP_518334
72	Brg21	RSp0218	*R. solanacearum*		GMI1000	NP_518339
73	Brg23	RSp0257	*R. solanacearum*		GMI1000	NP_521818
74	Brg31	RSp0099	*R. solanacearum*		GMI1000	NP_521660
74	Brg32	RSp0847	*R. solanacearum*		GMI1000	NP_522408
74	Brg30	RSp1024	*R. solanacearum*		GMI1000	NP_522585
75	Brg33	RSp0845	*R. solanacearum*		GMI1000	NP_522406
76	Brg34	RSp0879	*R. solanacearum*		GMI1000	NP_522440
77	Brg35	RSp0885	*R. solanacearum*		GMI1000	NP_522446
78	Brg37	RSp1022	*R. solanacearum*		GMI1000	NP_522583
79	Brg43	RSp1384	*R. solanacearum*		GMI1000	NP_522943
80	Brg44	RSp1130	*R. solanacearum*		GMI1000	NP_522691
81	Brg45	RSp1460	*R. solanacearum*		GMI1000	NP_523019
82	Brg48	RSp1388	*R. solanacearum*		GMI1000	NP_522947
83	SseF		*S. enterica*	typhimurium	LT2	NP_460369

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	Name [b]	Alt. name	*Strain*	pv.[c]	Isolate	Accession
84	SseG		*S. enterica*	typhimurium	LT2	NP_460370
85	SseI		*S. enterica*	typhimurium	LT2	AAL19985
86	SseJ		*S. enterica*	typhimurium	LT2	AE008772
87	SifB		*S. enterica*	typhimurium	LT2	NC_003197
88	SopB		*S. enterica*	typhimurium	LT2	NC_003197
89	PipB		*S. enterica*	typhimurium	LT2	NC_003197
90	SseE		*S. enterica*	typhimurium	LT2	NP_460367
91	SifA		*S. enterica*	typhimurium	LT2	AAL20153
92	SipA	STY3005	*S. enterica*	typhimurium	CT18	NP_457276
93	SipB	STY3008	*S. enterica*	typhimurium	CT18	NP_457279
94	SipC	STY3007	*S. enterica*	typhimurium	CT18	NP_457278
95	HopAJ1	HopPmaG	*P. syringae*	maculicola	ES4326	AF458045
95	HopAJ1*	PSPPH_0763	*P.syringae*	phaseolicola	1448A	AAZ34315.1
95	HopAJ2	PSPPH_4398	*P.syringae*	phaseolicola	1448A	AAZ34636.1
95	HopAJ2	Psyr_4357	*P. syringae*	syringae	B728a	AAY39387.1
95	HopAJ1*	PSPTO0852	*P.syringae*	tomato	DC3000	NP_790692.1
95	HopAJ2	PSPTO4817	*P.syringae*	tomato	DC3000	NP_794551.1
96	AvrRpt2		*P.syringae*	tomato	JL1065	L11355
97	HopAR1	AvrPphB	*P.syringae*	phaseolicola	race3	M86401
98	HrpZ1		*P.syringae*	carambola	HL1	AY372185
98	HrpZ1		*P.syringae*	apata		AF092879
98	HrpZ1		*P.syringae*	glycinia	race 4	L41862
98	HrpZ1		*P.syringae*	maculicola	M2	AY325899
98	HrpZ1		*P.syringae*	phaseolicola	1302A race4	AF268940
98	HrpZ1	PSPPH_1273	*P.syringae*	phaseolicola	1448A	AAZ34500.1
98	HrpZ1		*P.syringae*	pisi	race 1	AB018082
98	HrpZ1		*P.syringae*	pisi	race 2	AB018083
98	HrpZ1		*P.syringae*	pisi	race 3	AB018084
98	HrpZ1		*P.syringae*	pisi	race 4	AB018085
98	HrpZ1		*P.syringae*	syringae	61	L14775
98	HrpZ1	Psyr_1193	*P.syringae*	syringae	B728a	AAY36247.1
98	HrpZ1		*P.syringae*	syringae	LOB201	AB102727
98	HrpZ1		*P.syringae*	syringae	NV	AF031667
98	HrpZ1		*P.syringae*	tabaci	6605	AB049570
98	HrpZ1	PSPTO1382	*P.syringae*	tomato	DC3000	NP_791209
99	YscN		*Y. pestis*		KIM	NP_668159

Supplemental Table 1 Notes:
[a] **Bold font** indicates proteins families represented in Figure 3 of the main text.
[b] Proteins marked (*) are not translocated using full length fusions to truncated AvrRpt2 as an assay (1). Note that other members of some of these families are either not tested, or have been demonstrated to be translocated.
[c]Pathovar

Supplemental Table 1. Analysis: Proteins were identified as type III effectors based on other studies. *P. syringae* were either experimentally confirmed as translocated (1) or identified as type III effector candidates by a variety of computational methods (5). *R. solanacearum* GMI1000 proteins were found based on their *hrp*-dependent transcriptional regulation (2). 41 of these are predicted type III effectors and were included along with known *R. solanacearum* type III effector protein PopC. Other sources for type III effector protein sequences were: *Salmonella enterica* (8); *Yersinia pestis* (7); Xanthomonas spp. (6) and (3).

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