Review

The type III effectors of *Xanthomonas*

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SUMMARY

A review of type III effectors (T3 effectors) from strains of *Xanthomonas* reveals a growing list of candidate and known effectors based on functional assays and sequence and structural similarity searches of genomic data. We propose that the effectors and suspected effectors should be distributed into 39 so-called Xop groups reflecting sequence similarity. Some groups have structural motifs for putative enzymatic functions, and recent studies have provided considerable insight into the interaction with host factors in their function as mediators of virulence and elicitors of resistance for a few specific T3 effectors. Many groups are related to T3 effectors of plant and animal pathogenic bacteria, and several groups appear to have been exploited primarily by *Xanthomonas* species based on available data. At the same time, a relatively large number of candidate effectors remain to be examined in more detail with regard to their function within host cells.

INTRODUCTION

The genus *Xanthomonas* consists of a large group of plant pathogenic proteobacteria which, as a group, has considerable agricultural impact. Common diseases found in nature include the vascular wilts, cankers, leaf spots, fruit spots and blights. Despite the wide host range of *Xanthomonas*, individual strains can be highly restricted to particular host plants. *Xanthomonas* spp. infect hosts encompassing most of the major groups of higher plants, including 124 monocotyledonous and 268 dicotyledonous plant species (Hayward, 1993; Leyns *et al.*, 1984; Starr, 1983). Many xanthomonads require a type III secretion system (T3SS) for pathogenicity on plant hosts, and the requirement reflects the utilization of T3 effectors to mediate the processes of pathogen adaptation to specific host tissues, species and genotypes. T3 effectors are translocated into the host cells where they

interfere with host immunity responses or facilitate nutritional or virulence processes of the pathogen (Buttner and He, 2009). The diversity in tissue and host specificity of members of the genus is also undoubtedly reflected in the diversity of T3 effectors in a given pathovar or species. This review provides a catalogue of T3 effectors and candidate effectors that have been identified within *Xanthomonas* species, and is written as a prelude to a new wave of genomic sequence data and biochemical insight regarding the identification and function of T3 effectors in many plant pathogenic strains, pathovars and species. We aim to highlight the diversity of effectors in *Xanthomonas* species, recognizing the tremendous advances made in understanding T3 effector function in other bacterial disease complexes, including those of *Xanthomonas*, which are expertly reviewed in greater detail in this issue and elsewhere (Cunnac *et al.*, 2009; Lindeberg *et al.*, 2009).

CATALOGUE OF XANTHOMONAS EFFECTORS

The number of candidate and known T3 effectors of *Xanthomonas* stands at nearly 40 groups of related T3 effectors, as proposed here (Table 1). A more extensive list of candidates and updates can be found at <http://www.xanthomonas.org/t3e.html>, and information regarding updates or corrections to the data can be provided at the site. The effectors are grouped on the basis of sequence relatedness. The candidate status of an effector is based on the lack of functional secretion and/or translocation evidence. We have proposed the adoption of the Xop/Hop/Yop nomenclature, whilst recognizing that some T3 effectors are inexorably linked with the avirulence terminology. Thus, AvrBs1, AvrBs2 and AvrBs3 remain as named. The Xop designation originally was used to refer to proteins secreted in a T3SS-dependent manner and not strictly in reference to T3 effectors (Noel *et al.*, 2002). XopA was given to the name of the Hpa1 homologue from *X. campestris* pv. *vesicatoria* and therefore does not appear here as a T3 effector group (Noel *et al.*, 2002). Hpa1 (also referred to as HpaG) is a harpin-like protein that is also secreted in a T3SS-dependent manner, together with the curious pectic lyase-harpin fusion protein HrpW. All xanthomonads shown to possess a T3SS also have Hpa1/HpaG, but not all strains have a

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Table 1 Candidate and known T3 effectors of *Xanthomonas*.

Effector class*	Related proteins/synonyms	Xoo† MAFF311018	Xact 306	Xcv† 85-10	Xcc† ATCC33913	Xoo AXO1947†	Xcmu/ Xvm†	Xp 91-118†	Comments‡
AvrBs1	AvrA	N§	N	XCVd0104	XCC2100	N	N	N	A, V, U
AvrBs2		X000148	XAC0076	XCV0052	XCC0052	+	+	+	A, V, B
AvrBs3¶	Pth, TAL	17 genes	4 genes	Y** (P14727)	N	~8 genes	N	N	A, V, P, B
XopB (HopD1)		N	N	XCV0581	N	N	+	N	S, U
XopC	RSp1239	X003221	XAC1210/ XAC1209††	XCV2435 XCV1238/ XCV1237††	Y** (ACS12858)	+	+	+	A, C, S, B
XopD		N	N	XCV0437	XCC2896	N	N	+	A, C, V, P, B
XopE‡‡ (HopX)	AvrPphE AvrXccE1 HopPmaB	N	XAC0286 (E1) XAC3224 (E3) XACb0011(E2)	XCV0294(E1) XCV2280 (E2)	XCC1629 (E2)	N	N	+	A, C, S, B, M
XopF‡‡	Hpa4	X000103 (F1)	XAC2785 (F2)	XCV0414 (F1) XCV2942 (F2)	XCC1218 (F1)	+	+	+	C, U
XopG (HopH, HopAP)	AvrPtoH	X004258	N	XCV1298	XCC3258	N	+	N	A, S, B
XopH (HopAO)	AvrBs1.1 HopPtoD2	N	N	XCVd0105	XCC2099	N	N	N	A, S, B
XopI		X003626	XAC0754	XCV0806	N	+	+	+	A, S, B
XopJ (HopZ)	YopJ, AvrRxv AvrXv4, AvrBsT	N	N	XCV2156 XCV0471 Y** (AAD39255) Y** (AAG39033)	XCC3731	N	+	N	A, S, V, B, M
XopK		X001669	XAC3085	XCV3215	XCC2899	+	+	+	C, U
XopL		X001662	XAC3090	XCV3220	XCC4186	+	+	+	B
XopM§§				+					
XopN (HopAU)		X000315	XAC2786	XCV2944	XCC0231	+	+	+	A, C, V, P, B
XopO (HopK)	AvrRps4	N	N	XCV1055	N	+	+	+	A, C, U, B
XopP		X003222	XAC1208	XCV1236	XCC1247	+	+	+	A, C, U
XopQ (HopQ)		X004208	XAC4333	XCV4438	XCC1072	+	+	+	A, C, U
XopR		X004134	XAC0277	XCV0285	XCC0258	+	+	+	C, U
XopS§§				+					
XopT		X002210	NA, I	N	N	N	N	N	C, U
XopU		X002877	N	N	N	+	N	N	C, U
XopV		X003803	XAC0601	XCV0657	N	+	+	+	C, U
XopW		X000037	N	N	N	+	N	N	C, U
XopX		X004042	XAC0543	XCV0572	XCC0529 XCC0530	+	+	+	C, U, V
XopY		X001488	N	N	N	+	+	N	C, U
XopZ (HopAS)	AWR	X002402	XAC2009	XCV2059	XCC1975	+	+	+	C, U
XopAA (HopAE)	ECF, HolPsyAE	X002875	N	XCV3785 Y** (AY842297)	N	+	+	N	C, U, P
XopAB		X003150	N	N	N	+	+	+	C, U
XopAC	AvrAC	N	N	N	XCC2565	N	N	N	A, B
XopAD	skwp¶¶¶ (BAH47290)	X004145	XAC4213	XCV4315/XCV4314/ XCV4313††	N	+	+	+	U
XopAE	HpaF PopC	X000110	XAC0393	XCV0409/XCV0408	N	+	+	+	V, B
XopAF (HopAF)	AvrXv3	N	N	N	N	N	+	+	A, U
XopAG (HopG)	AvrGf1	N	Y** (ABB84189)	N	XCC3600	N	+	N	A
XopAH	AvrXccC AvrB, AvrC	N	N	N	XCC2109	N	N	N	V, S, C, M
XopAI (HopO1)		N	XAC3230	N	N	N	N	N	B, M
XopAJ	AvrRxo1	N	N	XCV4428	N	N	N	N	B
XopAK (HopK1)		N	XAC3666	XCV3786	N	N	+	N	A, U

*Xop groups with members in every strain presented here are shaded in grey.

†Data were derived from the sequenced genomes of *X. oryzae* pv. *oryzae* strain MAFF311018 (Ochiai *et al.*, 2005), *X. axonopodis* pv. *citri* strain XAC 306 (da Silva *et al.*, 2002), *X. campestris* pv. *vesicatoria* strain XCV 85-10 (Thieme *et al.*, 2005), *X. campestris* pv. *campestris* strain ATCC33913 (da Silva *et al.*, 2002), *X. oryzae* pv. *oryzae* AXO1947 (F. F. White, unpublished results), *X. vasicola* pv. *musacearum* and *X. perforans* 91-118 (J. B. Jones, unpublished results).

‡Codes are: A, translocation evidence based on Avr activity or Avr reporter fusion; B, biochemical or structural evidence for putative effector function (see Table 2 for more details); C, translocation based on Cya fusion; M, membrane localization; P, effector associated with phenotypic response in plant; S, protein is secreted to extracellular milieu in a type III secretion system (T3SS)-dependent manner; U, protein of unknown function; V, evidence for enhancement of pathogen populations in plant.

§Codes are: I, insertion or truncation of coding sequence; N, not identified; NA, not annotated; +, presence of single related open reading frame (ORF) in unannotated genomic sequence data; ++, presence of two related ORFs.

¶Numbers indicate number of related genes in each genome.

**Y, some members of the groups were identified in another strain of the same species or pathovar. AvrBsT and early chlorosis factor (ECF) found in Xcv tomato strain 75-3, AvrGf1 found in *X. citri* Aw strain. GENBANK accession number is given in parentheses.

††Slash (/) indicates reading frame shift within related sequence.

‡‡Subfamily based on sequence relatedness is indicated in parentheses for XopE and XopF.

§§Group assigned to candidate effector and sequence information is not presently available.

¶¶¶Accession number for protein from *Ralstonia solanacearum* in parentheses.

version of HrpW (Kim *et al.*, 2003; Noel *et al.*, 2002; Zhu *et al.*, 2000). Whether harpin-like proteins (harpin, Hpa1/HpaG and HrpW) function as virulence effectors or as accessory and translocation proteins is an important subject of research. However, the function of the harpin-like proteins remains unclear and will not be addressed here.

Torrent of genomic data is likely to expand the repertoire of T3 effectors of *Xanthomonas*

Candidate T3 effectors, historically, have been identified through the characterization of resistance (*R*) gene-mediated responses (Astua-Monge *et al.*, 2000a, b; Bonas *et al.*, 1989; Ciesiolka *et al.*, 1999; Ronald and Stascawicz, 1988; Rybak *et al.*, 2009; Swords *et al.*, 1996; Whalen *et al.*, 1993; Zhao *et al.*, 2004). Improvements in T3 effector assays that are independent of endogenous *R* gene responses have greatly facilitated the analysis of T3 effector translocation, and now many of the T3 effector candidates have supporting evidence for T3SS-dependent secretion and translocation to plant cells based on assays of avirulence reporter fusions (Greenberg and Vinatzer, 2003; Guttman *et al.*, 2002) and/or calmodulin-dependent adenylate cyclase (Cya) activity assays (Casper-Lindley *et al.*, 2002; Schechter *et al.*, 2004; Sory *et al.*, 1995). Consequently, additional candidate T3 effectors from *Xanthomonas*, which do not have associated host hypersensitive reactions (HRs), have come from functional assays of T3SS-dependent expression, secretion and screens of avirulence protein fusions on a genomic scale (Noel *et al.*, 2002, 2003; Roden *et al.*, 2004a; Thieme, 2008). More recently, candidates have been identified from genomic sequence data and subjected to further validation criteria (Collmer *et al.*, 2009). The annotated and assembled genomic sequences of eight strains of *Xanthomonas* are currently available, representing four pathovars (Lee *et al.*, 2005; Salzberg *et al.*, 2008; da Silva *et al.*, 2002; Thieme *et al.*, 2005). The genomic sequences of three closely related strains of *X. oryzae* pv. *oryzae* and *X. campestris* pv. *campestris*, respectively, have been determined (Ochiai *et al.*, 2005; Qian *et al.*, 2005; Salzberg *et al.*, 2008; Vorholter *et al.*, 2008). The T3 effector content of the different strains within a pathovar is similar with a few exceptions, and the data from a representative strain for each pathovar are presented (Table 1). In addition, nearly completed or draft sequences are now or will soon be available for nearly double the number of currently sequenced strains. Here, the draft sequences of three distinct strains were examined for candidate T3 effector genes on the basis of similarity searches, and were included as representative of the data expected in the near future. Strain AX01947 is an atypical *X. oryzae* pv. *oryzae* originally isolated in Africa (Gonzalez *et al.*, 2007; Gu *et al.*, 2004). *Xanthomonas vasicola* pv. *musacearum* (Xvm and, for-

merly, *X. campestris* pv. *musacarum*) is the causal agent of xanthomonas wilt on banana (Aritua *et al.*, 2008). *Xanthomonas perforans* 91–118 is one of a group of *Xanthomonas* species that cause bacterial spot of tomatoes and pepper (Jones *et al.*, 2000). Individual candidate effectors, derived from related sequence data, have been characterized (Jiang *et al.*, 2008; Xu *et al.*, 2008; Wang *et al.*, 2007). Furutani *et al.* (2009) recently provided T3SS-dependent translocation evidence for 16 candidate T3 effectors of *X. oryzae* pv. *oryzae*, including nine members of new T3 effector groups in *Xanthomonas* (Table 1, XopK, XopR, XopR-XopW and XopAB; Furutani *et al.*, 2009).

Subgrouping of T3 effectors

The 40 groups of Xops are likely to underestimate the variety of host physiological functions that are targeted by the T3SS virulence pathway in strains of *Xanthomonas*, as a number of groups have multiple members. Multimembered groups occur both as groups with relatively divergent members based on sequence relatedness, as found in the XopJ group, and as groups with highly conserved sequence relatedness but apparent proliferation of variants based on repetitive domains, as found in the large AvrBs3 family. The XopJ group, which is part of the broader YopJ family of related effectors in many animal and plant pathogenic proteobacteria with T3SS virulence systems, includes XopJ, AvrRxv, AvrBsT and AvrXv4. The XopJ group may encode members with variant enzymatic activities and target multiple host physiological pathways (Mukherjee *et al.*, 2007). Variations of the large AvrBs3 family, which is also referred to in the literature as the AvrBs3/PthA family, and the transcription activation-like (TAL) T3 effectors occur both between strains as well as within individual strains of *Xanthomonas* (Schomack *et al.*, 2006). Individual strains of *X. oryzae* pv. *oryzae* are known to contain 19 genes for TAL effectors, and one strain of *X. oryzae* pv. *oryzicola* contains 28 TAL effector genes (Ochiai *et al.*, 2005; Salzberg *et al.*, 2008; A. Bogdanove, Iowa State University, Ames, IA). Different TAL effectors target different host genes and are likely to affect different host physiological processes (White and Yang, 2009). Other effector groups with two or three related genes in individual strains include XopE, XopF, XopJ, XopX, XopAD and XopAE (Table 1, <http://www.xanthomonas.org/t3e.html>). Some of the groups have been further divided into subgroups based on phylogenetic data and, here, these subgroups have been assigned numbers to denote phylogenetic families within a group (e.g. XopF1 and XopF2, Table 1). Phylogenetic analyses remain to be performed on all groups, and additional groups are likely to be proposed. At the opposite end of the spectrum, some groups are defined by members that have been found in relatively few strains or pathovars. Effectors with limited distribution are XopB, XopD, XopT, XopU, XopW, XopAC, XopAF, XopAH, XopAJ, and XopAK.

XopAI	XAC3230	MGLCTSKPSVV---GSPVAGSPEHYLTHTAETPTPSTPSPEAPM
XopE2	XCV2280	MGLCSSKPSVV---GSPVAGSPEHYLTHTAETPTPSTPSPEAPM
XopE3*	XACb0011	MGLCSSKPSVA---GSPVAGSPEHYLTHTEETPTPSTPSPEAPM
XopE1	XAC0286	MGLCVSRPATS---GSSVAAASPEQHPNPASEQVTPHTPSVPETSV
XopE1	XCV0294	MGLCISKPAMS---GSSVAAASPEHPESPASEQVTPATP-LPEPSV
XopE1	XCC1629	MGLCVSKPSV-----AGSPDHYATHALEQTPPSTPSSEAPM
HopPmaB*		MGLCVSK-----GST-ASSPQHYAVRYTEQVTPS-PSPSVAST
XopJ	XCV2156	MGLCVSKPSV-----AGSPEHYAAHVAEQATPSEEGSGTPAQ
XopJ _{xcc}	XCC3731	MGLCNKS-VAGSVVGSPITATSPHEHYPTSATEQTPFRPSSPEASM
AvrBst*		MGLCTSKPSVV---GSPVAGSPEQYLSHTTEQTPSTPSPDAPM*
*		
XopAI	XAC3230	SPSLHGLAALGSPRASSSPRPLSLVELNTSDLIKQKKQLWQ....
XopE2	XCV2280	SPSLHGLVALGSSGTRRGRFRQPTLQPHEVQQAAYQLGMRLS....
XopE3*	XACb0011	SPSLHGLVALGSSGTRRDRFRQPTLQPHEVQQAAYQLGMRLS....
XopE1	XAC0286	PSPQLGLPTRCVPRARRGRSGKPTLQPHEVQQAAYQLGMRLS....
XopE1	XCV0294	PSPQLGLARLGAAARARRRRSGKPTLQPHEVQQAAYQLGMRLS....
XopE1	XCC1629	SPSLHGLPSLGARRARR-AGAGPTLQPHEVQQAAYQLGMRLN....
HopPma1*		SPSLHDSSTAIGSPLARRS----SVLLPHEVQQAAYQLAMRLN....
XopJ	XCV2156	ATSSYSATDPALQGLARRGKKVDLSRSGLPERVALKTKLLAN....
XopJ _{xcc}	XCC3731	SPSLHDLAALGSPPRARRARSGRAASPSGTGIMAGRVSFPS....
AvrBst*		SPSLHGLVALGSLGTRRAF*

Fig. 1 A variety of Xop proteins of different groups share N-terminal domains. The highlighted text indicates related Xop proteins based on their C-terminal regions. Yellow, XopAI group; grey, XopE group and related *Pseudomonas syringae* T3 effector HopPmaB (AAL84240); blue, XopJ-related proteins. Asterisk indicates the G residue for myristoylation (Nimchuk *et al.*, 2000).

Genetic flux between pathogenic bacteria

The T3 effectors of *Xanthomonas* reflect the robust exchange of genetic material between bacteria. Slightly less than one-half of the groups (15) are related to candidate T3 effectors of *Pseudomonas syringae* pathovars to varying degrees (Table 1, <http://www.xanthomonas.org/t3e.html>), and many have related members in *Ralstonia solanacearum* and *Acidovorax avenae* ssp. *citrulli*, the members of which, with some exceptions, are not presented here. Eleven groups have a homologue or, at least, partial relatedness in every strain of *Xanthomonas* that has been sequenced (Table 1). However, the homologues may not be functional in all strains. For example, the lone XopF open reading frame (ORF) from *X. axonopodis* pv. *citri* lacks the N-terminal coding sequence (Table 1, XAC2785) and is likely to represent a truncated gene. A number of genes for T3 effectors, as predicted from genomic sequences, require re-annotation because of improper or unknown start sites, as noted for XopE members (Thieme *et al.*, 2007). The T3 effectors of *Xanthomonas* also include chimeric proteins, which can confuse classification schemes. XAC3230 is the sole member of the proposed XopAI group and contains a 43-amino-acid N-terminal domain that is also found in the N-termini of effectors in groups XopE and XopJ (Fig. 1). The XAC3230 N-terminal domain also contains a myristoylation motif, which was previously identified in several T3 effectors of *P. syringae*, indicating that effectors with this N-terminal domain are targeted to host cellular membranes. Evidence for the myristoylation of XopJ, XopE1 and XopE2 of *X. campestris* pv. *vesicatoria* has been demonstrated by the loss of membrane localization on mutagenesis of the conserved G2 residue in each of the proteins (Thieme *et al.*, 2007). Thus, N-terminal domain shuffling may not only be involved in the recruitment of new effectors, but also may

allow modifications to effector targeting within the host cell (Stavrínides *et al.*, 2006).

New classes of T3 effectors await biochemical analysis

Functional analyses of candidate T3 effector translocation and effects on host physiology involving *Xanthomonas* species have not kept pace with the genome sequence analyses, and some effector groups have little or no experimental support other than evidence based on relatedness to effectors of other pathogens. These groups include the aforementioned XopL, XopAD, XopAE and XopAI. XopAI, in addition to the high similarity to the N-terminal sequences from known T3 effectors, is weakly related to HopO1 (Almeida *et al.*, 2009). However, both XopAI and HopO1 share a VIP2 ADP-ribosylation motif, indicating that the proteins may have conserved structural features. XopL and XopAE both include members with leucine-rich repeats (LRRs), a motif also present in XopAC. XopAE represents the HpaF proteins, the genes for which are found within the T3SS (Hrp) gene clusters of most strains, with the exception of *X. campestris* pv. *campestris* (Kim *et al.*, 2003). The genes for HpaF are co-regulated with T3SS genes, and mutations in *hpaF* from *X. axonopodis* pv. *glycines* cause reduced virulence (Kim *et al.*, 2003). No evidence has been reported for T3SS-dependent secretion of HpaF. A member of XopG was originally identified as part of the putative HrpX regulon in *X. campestris* pv. *campestris* ATCC33913 (da Silva *et al.*, 2002). Members are related to the predicted T3 effectors HopH1 and HopAP1 of *P. syringae* species (Almeida *et al.*, 2009; Feil *et al.*, 2005) and RSp0572 of *R. solanacearum* GMI1000 (Salanoubat *et al.*, 2002). The XopAD group is noteworthy and solely based on a multigene family of putative T3 effectors from *R. solan-*

acearum, which are known as the SKWP proteins because of the presence of a 42-amino-acid repeat with the short consensus amino acid segment SKWP (Mukaihara and Tamura, 2009). Related SKWP proteins are encoded by a broad range of plant pathogenic bacteria, including one strain of *P. syringae* (*P. syringae* pv. *phaseolicola* 1448A). All of the strains discussed here have members of XopAD with the exception of *X. campestris* pv. *campestris*.

ELICITOR-MEDIATED IMMUNITY

Many T3 effectors in *Xanthomonas* undoubtedly function to suppress so-called pathogen-associated molecular pattern (PAMP)-triggered immune (PTI) responses, similar to the suppression observed by the T3 effectors of other plant pathogenic bacteria (Boller and Felix, 2009; Boller and He, 2009; Hotson and Mudgett, 2004; Kim *et al.*, 2008, 2009; Metz *et al.*, 2005). A variety of PTI responses have been characterized, and many are known to be triggered by pathogen recognition receptors (PRRs) on the plant cell surface. PRRs include the receptor-linked kinase (RLK) family, the best studied of which are the PAMP receptors for flagellin and elongation factor Tu (EF-Tu), namely FLS2 and EFR. PRRs, on perception of the elicitors, signal a variety of host defence responses, which have been shown to be suppressed by a variety of T3 effectors from *P. syringae* species at a variety of steps in the signalling pathways (Boller and He, 2009). The RLK family includes *Xa21*, which was the first *R* gene to be cloned from rice, and provides broad resistance against many strains of *X. oryzae* pv. *oryzae*, the agent of bacterial blight (Song *et al.*, 1995). Evidence indicates that the *Xa21* RLK serves as a PAMP signalling function, similar to FLS2 and ERF, with the exception of the specificity of the elicitor. The elicitor for *Xa21*-mediated resistance is hypothesized to be an extracellular modified peptide, and, perhaps worth re-emphasizing here, not the product of the T3SS pathway, despite the fact that the hypothesized elicitor has historically been referred to as AvrXa21 (Lee *et al.*, 2006). A second *R* gene, *Xa26*, which gives broad resistance to *X. oryzae* pv. *oryzae*, also encodes an RLK (Sun *et al.*, 2004). With regard to PAMPs, flagellin and EF-Tu, the cognate elicitors for FLS2 and EFR, respectively, are produced by species of *Xanthomonas*, and pretreatment of plants with the conserved flg22 portion of bacterial flagellin enhances the resistance of *Arabidopsis* to *X. campestris* pv. *campestris* (Sun *et al.*, 2006). Additional compounds have been purified from *Xanthomonas* that elicit defence-related responses, including peptidoglycan polymers and lipopolysaccharides (LPSs), and other compounds may have activity in PTI in response to infections by *Xanthomonas* species, particularly in specific hosts (Erbs *et al.*, 2008; Keshavarzi *et al.*, 2004; Silipo *et al.*, 2005).

Suppression of elicitor-mediated immunity

Xanthomonas species have been known for some time to suppress host defence responses in a T3SS-dependent manner and, more recently, specific T3 effectors have been shown to suppress defence responses and defence-related gene expression (Brown *et al.*, 1995; Keshavarzi *et al.*, 2004). The presence of elicitation-competent flagellin genes in *X. campestris* pv. *campestris* has been shown not to control the degree of virulence, and the absence of FLS2, the RLK receptor for flg22, also has no effect on disease, indicating that *X. campestris* pv. *campestris* suppresses PTI as mediated by FLS2 and EFR (Sun *et al.*, 2006). The T3SS of *X. oryzae* pv. *oryzae* has been shown to be required for the suppression of rice defence responses that are elicited by the application of extracellular degradative enzymes, indicating that elicitors of PTI include products of microbial enzymes acting on host substrates (Jha *et al.*, 2007). At the same time, *Xa21/Xa26*-mediated resistance responses, as broad resistances in rice, are obviously not suppressed by many extant strains of *X. oryzae* pv. *oryzae*. If *X. oryzae* pv. *oryzae* strains can suppress PRR-mediated PTI, in general, specific PRRs have apparent signalling pathways that circumvent T3 effector function.

Specific T3 effectors of *X. campestris* pv. *vesicatoria* have also been associated with the suppression of various PTI and host defence responses. XopX was identified in a search for a nonhost avirulence gene and was found to increase the susceptibility of *Nicotiana benthamiana* to both *Xanthomonas* and *Pseudomonas* species (Metz *et al.*, 2005). The T3 effectors XopN and XopD have also been shown to reduce PTI and host developmental pathways that may play a role in host defence (Kim *et al.*, 2008, 2009). XopD suppresses the expression of a variety of defence- and senescence-related genes in tomato and is associated with lower levels of salicylic acid (SA) and delayed symptom development in infected tissue (Kim *et al.*, 2008). XopN also suppresses defence-related gene expression in tomato (Kim *et al.*, 2009); in a heterologous assay using *P. syringae*, XopN suppressed callose deposition in both tomato and *Arabidopsis*. XopJ has recently been demonstrated to suppress defence-related callose deposition and, more specifically, host protein extracellular secretion (Bartetzko *et al.*, 2009).

Suppressors of effector-triggered immunity

T3 effectors are best known for specific T3 effectors that are recognized by host defence surveillance systems and elicit a rapid HR, which has been defined as effector-triggered immunity (ETI) to distinguish it from PTI-triggered immunity. T3 effectors are also known to suppress *R* gene-mediated defence, and some evidence suggests that some T3 effectors of *Xanthomonas* are involved in ETI suppression (Rosebrock *et al.*, 2007). AvrBsT, a

member of the XopJ/YopJ family, triggers an HR in a single landrace of *Arabidopsis* with a recessive mutation in a gene for carboxylesterase, which was named *SUPPRESSOR OF AVRBS1-ELICITED RESISTANCE1* (SOBER). A model has been proposed that, in the absence of esterase activity, AvrBsT can suppress recognition by a host *R* gene product. The robust *R* gene responses to AvrXa10 and AvrXa7 of *X. oryzae* pv. *oryzae* in rice cultivars containing the *R* genes *Xa10* and *Xa7*, respectively, are suppressed when the corresponding genes for the effectors are introduced into the closely related pathogen *X. oryzae* pv. *oryzicola*.

VIRULENCE AND DISEASE SYMPTOMATOLOGY ASSOCIATED WITH T3 EFFECTORS

All strains of *Xanthomonas* that possess a consensus *Xanthomonas* T3SS pathway are essentially nonpathogenic if the T3SS pathway is inactivated, indicating that, at least *in toto*, the T3 effectors have critical functions in facilitating host parasitism. At the same time, mutations of individual effector genes often do not result in a change in the virulence phenotype or have a relatively small effect, which may reflect the aspects of the experimental context and/or redundancy of effector function (Castaneda *et al.*, 2005). However, whether suppressing elicitor-triggered immunity or providing some other beneficial function, a growing number of T3 effectors of *Xanthomonas* have been associated with enhanced virulence of strains and aspects of disease symptomatology (Table 1). AvrBs2 was one of the first effectors to contribute to virulence, which, in this example, is based on the ability to support increased numbers of bacteria within host tissue (Gassmann *et al.*, 2000; Kearney and Staskawicz, 1990). Curiously, the gene for AvrBs2 is found in all of the strains represented here and has only been shown to contribute to virulence in *X. campestris* pv. *vesicatoria*. Four T3 effectors of *X. campestris* pv. *vesicatoria* (AvrBs1–4) were all found to have varying degrees of contributions to pathogen fitness in field situations (Wichmann and Bergelson, 2004). An extreme requirement for an individual T3 effector is found in the diseases caused by strains of both *X. axonopodis* pv. *citri* and *X. oryzae* pv. *oryzae*. In the last two species, mutations in single TAL effector genes (*pthA* or *pthXo1*, respectively) can have a debilitating effect on both the ability of the pathogen to spread and to elicit disease symptomatology (Swarup *et al.*, 1991; Yang and White, 2004). The TAL effectors constitute a large family of closely related T3 effectors and many affect virulence and/or disease symptomatology. PthA, which is the critical TAL effector from *X. axonopodis* pv. *citri*, is required for host cell hypertrophy and high bacterial populations (Swarup *et al.*, 1991). The phenotype is also observed on *in planta* expression of the gene and in the absence of bacteria (Duan *et al.*, 1999). A similar behaviour is observed with the gene for

AvrBs3 from *X. campestris* pv. *vesicatoria* (Marois *et al.*, 2002). Host cell hypertrophy is not observed in rice. However, lesions, which in the rice system, occur as chlorotic and desiccated leaf tissue, are severely limited in the absence of a major TAL effector for virulence (Bai *et al.*, 2000; Yang and White, 2004; Yang *et al.*, 2000). TAL effectors of *X. campestris* pv. *malvacearum* and *X. oryzae* pv. *oryzae* are associated with increased water soaking, a phenotype often associated with bacterial infections of plants. One benefit of TAL effectors to bacteria may be an enhancement of the release of bacteria from infected tissue. Increased numbers of bacteria in leaf washings were demonstrated for *X. campestris* pv. *malvacearum* with AvrB6 in cotton and *X. axonopodis* pv. *citri* with PthA in citrus when compared with tissue infected by strains without effectors (Yang *et al.*, 1994). In pepper (AvrBs3) and cotton (AvrB6), significant increases in bacterial populations have not been detected in the presence of effectors. However, enhanced release could have a fitness benefit in field infections, and fitness benefits to AvrBs3 were observed in field experiments (Wichmann and Bergelson, 2004). The importance of TAL effector function in rice is supported by the deployment of recessive resistance (Lee *et al.*, 2003). The *R* gene *xa13* specifically interferes with the function of the TAL effector PthXo1, indicating that interference in TAL effector function has proven to be effective in providing resistance against bacterial blight of rice (Yang *et al.*, 2006).

Both XopD and XopN of *X. campestris* pv. *vesicatoria* have been shown to contribute to virulence and disease symptomatology in tomato (Kim *et al.*, 2008, 2009; Roden *et al.*, 2004a). Plants infected with strains without XopD show greater leaf tissue necrosis, indicating that XopD is involved in the suppression of host-mediated tissue degeneration, which may represent a defence reaction to infection (Kim *et al.*, 2008). Strains without XopN, however, possess lower bacterial populations and less necrosis and senescence at later time points after inoculation (Kim *et al.*, 2009). Loss of the XopN homologue in *X. campestris* pv. *campestris* also results in reduced disease in radish, and genes for members of the family are widely distributed in *Xanthomonas* (Jiang *et al.*, 2008). Another widely distributed T3 effector that affects host tissue responses is early chlorosis factor (ECF), which was labelled XopAA (Morales *et al.*, 2005). ECF was isolated by a complementation strategy, whereby strains with a reduced ability to induce chlorosis were converted to a greater ability to induce chlorosis by the transfer of the gene for ECF. ECF had no measurable effect on bacterial populations. Other candidate T3 effector groups with members that have reported effects on virulence and disease symptomatology include XopJ (AvrXv4; Roden *et al.*, 2004b), XopX (Metz *et al.*, 2005), XopAE (HpaF; Kim *et al.*, 2003) and XopAH (AvrXccC; Wang *et al.*, 2007).

Table 2 Evidence for biochemical and biological functions of T3 effectors from *Xanthomonas*.

Effector group*	Biochemical or structural motifs†	Host factor‡	Reference
AvrBs2	Glycerolphosphoryl diester phosphodiesterase		Kearney and Staskawicz, 1990; Swords <i>et al.</i> , 1996
AvrBs3/PthA	Site-specific DNA binding; nuclear localization; transcription activation domain	<i>Os8N3</i> (r); <i>OsTFX1</i> ; <i>OsTFIIAγ1</i> ; <i>upa20</i> (p)	Van den Ackerveken <i>et al.</i> , 1996; Kay <i>et al.</i> , 2007; Romer <i>et al.</i> , 2007; Sugio <i>et al.</i> , 2007; Yang <i>et al.</i> , 2006; Zhu <i>et al.</i> , 1998
XopC	Haloacid dehalogenase hydrolase and phosphoribosyl transferase domain		Noel <i>et al.</i> , 2003
XopD	Cysteine SUMO C48 protease; DNA binding; nuclear localization; EAR motif		Chosed <i>et al.</i> , 2007; Hotson <i>et al.</i> , 2003; Kim <i>et al.</i> , 2008; Noel <i>et al.</i> , 2002
XopE (HopX)	Transglutaminase family		Nimchuk <i>et al.</i> , 2007; da Silva <i>et al.</i> , 2002; Thieme <i>et al.</i> , 2007
XopG (HopH1 and HopAP1)	M27 zinc protease (clostridial toxin)		Ochiai <i>et al.</i> , 2005; Salzberg <i>et al.</i> , 2008; da Silva <i>et al.</i> , 2002
XopJ (HopJ1) [AvrBsT, AvrXv4, AvrRxv]	Acetyltransferase, C55 cysteine ubiquitin-like protease	14-3-3 protein (t)	Bartetzko <i>et al.</i> , 2009; Noel <i>et al.</i> , 2003; Roden <i>et al.</i> , 2004b; Thieme <i>et al.</i> , 2007; Whalen <i>et al.</i> , 2008
XopH (HopAO1)	Tyrosine phosphatase		Bretz <i>et al.</i> , 2003; Espinosa <i>et al.</i> , 2003
XopN (HopAU1)	ARM/HEAT repeat	TARK1 (t), 14-3-3 (t)	Kim <i>et al.</i> , 2009; Roden <i>et al.</i> , 2004a
XopQ (HopQ)	Nucleoside hydrolase		Wei <i>et al.</i> , 2007
XopAA (HopAE1)	Colicin Ia C8 and C9 domains; ECF		Morales <i>et al.</i> , 2005
XopAC	FIDO domain (AMPylation) PF02661; LRR, VHR		Kinch <i>et al.</i> , 2009; Xu <i>et al.</i> , 2008
XopAH	FIDO (AMPylation) PF02661		Kinch <i>et al.</i> , 2009; Wang <i>et al.</i> , 2007
XopAI (HopO1)	VIP2; ADP-ribosyltransferase		da Silva <i>et al.</i> , 2002
XopAJ [AvrRx]	Thiol protease, ATP/GTP binding		Zhao <i>et al.</i> , 2004

*Names of related effector groups from *Pseudomonas syringae* are given in parentheses, and names of other *Xanthomonas* effectors in the group are given in square brackets.

†Abbreviations (noted characteristics and inclusion do not imply confirmation of activity or function): ARM/HEAT, armadillo/Huntington, elongation factor 3, PR65/A, TOR domain; EAR, ethylene receptor factor-associated amphiphilic repression; ECF, early chlorosis factor; FIDO, fic, doc, AvrB domain; LRR, leucine-rich repeat; SUMO, small ubiquitin modifier protein; VHR, vascular hypersensitivity in *Arabidopsis* landrace Col-0.

‡Host genes or proteins that are associated with effector by binding, genetic or other experimental evidence. Abbreviations: (p), pepper; (r), rice; TARK, tomato atypical receptor kinase; (t), tomato.

BIOCHEMICAL AND EXPERIMENTAL CLUES TO T3 EFFECTOR FUNCTION

A variety of putative structural motifs contained in the primary structure of T3 effectors have provided insights into the putative biochemical function of T3 effectors. Fifteen of the effector classes of *Xanthomonas* have structural motifs that have provided clues to the function of some effectors. Twelve of the 15 indicate the possibility of enzymatic function (Table 2). The XopJ (YopJ) group has been reviewed in considerable detail (Mukherjee *et al.*, 2007; Hotson and Mudgett, 2004). Others that have received less focus in *Xanthomonas* include the two groups (XopAC and XopAH) with the recently described Fido/AvrB domain (Kinch *et al.*, 2009), XopAI (XAC3230) containing a VIP2 actin ADP-ribosylation domain (Han and Tainer, 2002; Han *et al.*, 2001) and XopG with a zinc protease motif similar to the active site of clostridial neurotoxins (Lalli *et al.*, 2003; Turton *et al.*, 2002) (Table 2). Considerable progress into the specific functions of individual T3 effectors from *Xanthomonas* and their interaction with host plants has also provided insight into T3 effector function, and highlights from some of the Xop groups are provided below.

AvrBs3

The AvrBs3 group represents a remarkably large family of closely related T3 effectors of *Xanthomonas*, which, *in toto*, are referred to here as the TAL effector family. A variety of TAL effectors are associated with biological effects, including virulence of the pathogen and disease symptoms (Table 3). All TAL effectors have a characteristic overall structure with the most distinguishing feature of the individual effectors being a central repetitive region containing varying numbers of near-identical repeats of 34 or 35 amino acids. The N-terminal and C-terminal portions of the proteins are highly conserved, and differences between effectors, in terms of their biological activity, are related to the particular repetitive region. For example, the type member AvrBs3 has 17.5 repeats, whereas AvrXa7 from *X. oryzae* pv. *oryzae* has 26.5 repeats (Bonas *et al.*, 1989; Yang *et al.*, 2000). Shuffling of specific repetitive regions can disrupt avirulence specificity (Herbers *et al.*, 1992; Yang *et al.*, 2005), and, in general, the repetitive regions can be swapped between effectors, and the specific activity associated with the effector will be associated with whichever has the original repetitive region (Yang and White, 2004; Yang *et al.*, 2000). The structural role

Table 3 Transcription activation-like (TAL) T3 effectors of *Xanthomonas* with associated phenotypes.

Effector	Tag*	Species†	Phenotypes‡, host target genes, (host species)§	Reference
Apl1	BAA37119	Xac, NA-1	H (ci)	Kanamori and Tsuyumu, 1998
Apl2	BAA37120	Xac, NA-1	Hw (c)	Kanamori and Tsuyumu, 1998
Avrb6	AAB00675	Xcm, XcmH	W, R, b6 (co)	De Feyter <i>et al.</i> , 1993; Yang <i>et al.</i> , 1994
AvrBs3	P14727	Xcv, 71-21	H, Bs3 (p), upa20 (p)	Bonas <i>et al.</i> , 1989; Kay <i>et al.</i> , 2007; Romer <i>et al.</i> , 2007
AvrBs4	CAA48680	Xcv, 82-8	Bs4 (t)	Bonas <i>et al.</i> , 1993; Schornack <i>et al.</i> , 2004
AvrHah1	ABP97430	Xg XV444	34 and 35¶, W (in pepper in Xcv), Bs3	Schornack <i>et al.</i> , 2008
AvrXa7	AAF98343	Xoo, PXO86, T7174, KXO85	Vma, Xa7 (r)	Bai <i>et al.</i> , 2000; Hopkins <i>et al.</i> , 1992; Ochiai <i>et al.</i> , 2005; Yang and White, 2004; Yang <i>et al.</i> , 2000
AvrXa10	AAA92974	Xoo, PXO86	Xa10 (r)	Hopkins <i>et al.</i> , 1992
AvrXa27	AAV54168	Xoo, PXO99 ^A , others	Xa27 (r)	Gu <i>et al.</i> , 2005
Hax2	AAV43358	Xca, 5	35¶, CN	Kay <i>et al.</i> , 2005
Hax3	AAV43359	Xca, 5	Bs4	Kay <i>et al.</i> , 2005
Hax4	AAV43360	Xca, 5	CN, Bs4	Kay <i>et al.</i> , 2005
HssB3.0	BAF46269	Xac, KC21	SHV	Shiotani <i>et al.</i> , 2007
PthA-KC21	BAF46271	Xac, KC21	H	Shiotani <i>et al.</i> , 2007
PthA	AAC43587	Xac	Vma,H,R	Swarup <i>et al.</i> , 1991
PthA*	ABO77780	Xac, Xc270 (A*)	H	Al-Saadi <i>et al.</i> , 2007
PthAW	ABO77779	Xac, X0053 (A ^W)	H	Al-Saadi <i>et al.</i> , 2007
PthB	NP_942641	Xcau, B96	H	Al-Saadi <i>et al.</i> , 2007
PthC	ABO77782	Xcau, C340	H	Al-Saadi <i>et al.</i> , 2007
PthN	AAB69865	Xcm, XcmN	W	Chakrabarty <i>et al.</i> , 1997
PthXo1	ACD58243	Xoo PXO99 ^A	Vma, Os8N3 (r)	Yang <i>et al.</i> , 2006
PthXo2	AAS46026	Xoo PXO71, T7174	Vma	Yang and White, 2004
PthXo3	AAS46027	Xoo, PXO61	Vma	Yang and White, 2004
PthXo6	ACD58920	Xoo, PXO99 ^A	Vm, OsTFX1 (r)	Sugio <i>et al.</i> , 2007
PthXo7	ACD57198	Xoo, PXO99 ^A	Vw*, OsTFIIAγ1 (r)	Sugio <i>et al.</i> , 2007
PthXo8	ACD60557	Xoo, PXO99 ^A	Vm, small RNA processing (r)	B. Yang and F. F. White, unpublished results

*Accession number for amino acid sequence.

†Pathovar designations used at the time of publication. Pathovar abbreviations: Xac, *X. axonopodis* pv. *citri*; Xca, *X. campestris* pv. *armoraciae*; Xcau, *X. citri* pv. *aurantifolii*; Xcm, *X. campestris* pv. *malvacearum*; Xcv, *X. campestris* pv. *vesicatoria*; Xg, *X. gardeneri*; Xoo, *X. oryzae* pv. *oryzae*.

‡Abbreviations: CN, enhanced chlorosis and necrosis of tissue; H, hypertrophy, cell enlargement; Hw, weak hypertrophy; R, increased bacterial numbers released from lesion; SHV, suppression of hypertrophy and virulence; Vm, moderate virulence effect; Vma, major enhancement of virulence on the basis of increased bacterial populations in plant; Vw, weak virulence; W, increase in water-soaked appearance of tissue. In Xoo, an increase in virulence is also accompanied by increased lengths of leaf lesions.

§Host species abbreviations: (c), citrus; (co), cotton; (p), pepper; (r), rice; (ra), radish (*Rhapanus sativus*); (t), tomato.

¶34, repetitive domain consisting of individual 34 amino acid repeats; 35, individual 35 amino acid repeats.

played by the repetitive region in the function and specificity of individual effectors is unknown, but has been proposed to determine the specific DNA sequence that is bound by the protein within the promoter of targeted host genes (Kay *et al.*, 2009; Romer *et al.*, 2009). The conserved C-terminal portion of TAL effectors contains potent nuclear localization signal (NLS) motifs, as well as a robust acidic transcription activation domain (AD), both of which are essential for pathogen virulence and associated effects on host disease symptoms (Szurek *et al.*, 2001; Van den Ackerveken *et al.*, 1996; Yang and Gabriel, 1995; Yang *et al.*, 2000; Zhu *et al.*, 1999). In cases in which there is a corresponding *R* gene, the NLS and AD are also required for *R* gene-mediated ETI (Gu *et al.*, 2005; Van den Ackerveken *et al.*, 1996; Yang *et al.*, 2000; Zhu *et al.*, 1998). TAL effectors induce the expression of specific host genes which, in the case of virulence, facilitate pathogen spread and disease (Yang *et al.*, 2006; Sugio *et al.*, 2007; Kay *et al.*, 2007).

Based on the recent results with AvrBs3, TAL effectors function as transcription factors that bind specific DNA elements in the host nucleus and induce the expression of targeted host genes (Kay *et al.*, 2007). AvrBs3 specifically binds to a DNA sequence in a number of genes, one of which, *upa20* (up-regulated by AvrBs3 20), encodes a basic helix–loop–helix transcription factor, which is responsible for the hypertrophy of the host cells. Ectopic expression of *upa20* is sufficient to cause host cell hypertrophy. The same AvrBs3-specific sequence is found in the promoter of the *Bs3* gene for resistance, resulting in the expression of *Bs3* in the presence of AvrBs3 (Romer *et al.*, 2009). Changes in the TAL effector binding element can result in a loss of TAL effector responsiveness. Specifically, the two alleles *Bs3* and *Bs3-E* of pepper differ by a 13-bp deletion (Romer *et al.*, 2007). Each element is bound by a corresponding variant, in this case AvrBs3, and a rearranged repetitive domain derivative named AvrBs3Δrep16, respectively, and the binding elements overlap by

11 bases (Romer *et al.*, 2009). Thus, the repetitive domain is probably involved in DNA binding specificity, and other TAL effectors may bind specific DNA elements in the respective host genomes.

The major TAL effector PthXo1 directs the expression of the rice gene *Os8N3*, a member of the nodulin3 protein family (Yang *et al.*, 2006). Failure to induce *Os8N3* results in resistance to infection unless an alternative TAL effector gene is present (Yang *et al.*, 2006). The recessive resistance gene *xa13* represents a set of *Os8N3* alleles that do not respond to compatible strains of the pathogen and, specifically, to PthXo1 (Chu *et al.*, 2006; Yang *et al.*, 2006; Yuan *et al.*, 2009). Silencing of *Os8N3* expression also results in resistance to strains of *X. oryzae* pv. *oryzae* that rely, specifically, on PthXo1 for virulence (Chu *et al.*, 2006; Yang *et al.*, 2006). However, *Os8N3* has an apparent function in pollen development and, possibly, in other aspects of fertility, and resistant plants produced by gene silencing suffer from low fertility (Chu *et al.*, 2006). Thus, the *xa13* alleles represent alterations that prevent or reduce sensitivity to PthXo1, whilst maintaining sufficient *Os8N3* expression patterns for development. *Os8N3* has been proposed to represent a class of polymorphic loci called susceptibility, or *S*, genes, with, in this case, PthXo1 as the cognate effector, in analogy with the *R* genes (Yang *et al.*, 2006). *S* genes are host loci where variations occur within the targets of the type III effectors, whereas many *R* genes have been proposed to guard or mimic type III effector targets (Hogenhout *et al.*, 2009). Alternative TAL effectors exist that overcome *xa13* resistance, and *Os8N3* induction is not involved in their ability to induce a state of host susceptibility (Yang *et al.*, 2006). Three of these effectors are AvrXa7, PthXo2 and PthXo3, and the analyses of the host responses and rice varieties are predicted to reveal an assortment of *S* genes, as well as *R* genes, and possible corresponding recessive resistance alleles that have arisen in rice evolution corresponding to TAL effectors (Yang *et al.*, 2006).

Three TAL effectors from a single strain of *X. oryzae* pv. *oryzae*, in addition to PthXo1, have been shown to provide contributions to virulence, and two are known to be associated with the elevated expression of two host genes distinct from *Os8N3* (Table 3). PthXo6 directs the elevated expression of a host gene named *OsTFX1*, which encodes a member of the large bZip family of transcription factors in plants (Sugio *et al.*, 2007). A mutant of strain PXO99^A in *pthXo6* suffered a reduction of virulence, although not to the same extent as the loss of genes for PthXo1 or any of the other major TAL effectors. In turn, ectopic expression of *OsTFX1* in rice abrogated the need for PthXo6 in the pathogen, but did not make the plants more susceptible to PXO99^A (Sugio *et al.*, 2007). The function of *OsTFX1* in a normal plant is unknown. bZip transcription factors are involved in the regulation of many cellular processes, and *OsTFX1* may induce or repress one or more downstream genes that are involved in host susceptibility or resistance, respectively. Ectopic expression of

the gene, other than compensating for the presence of *pthXo6* in the pathogen, did not cause any phenotypic abnormalities in the transgenic plants (Sugio *et al.*, 2007). All strains of *X. oryzae* pv. *oryzae* that were examined were capable of inducing *OsTFX1*, supporting the hypothesis that the ability to induce *OsTFX1* provides an important component to bacterial fitness in the environment.

PthXo7 is associated with the elevation of *OsTFIIAγ1* and has only been found in *X. oryzae* pv. *oryzae* strain PXO99^A (Sugio *et al.*, 2007). Loss of PthXo7 from PXO99^A has no measurable effect on strain virulence in susceptible rice plants. A small effect on disease symptomatology was found on transfer to strain PXO86, and the resulting strain was tested on rice containing the recessive resistance gene *xa5*, suggesting that PthXo7 may be an adaptation to specific host genotypes (Sugio *et al.*, 2007). PXO86 is incompatible on rice containing *xa5*, which, like *OsTFIIAγ1*, encodes a form of the small subunit of TFIIAγ (Iyer and McCouch, 2004; Jiang *et al.*, 2006). The *xa5* version does not support the growth of PXO86, and the elevation of *OsTFIIAγ1* was hypothesized to supply a form of TFIIAγ that facilitates bacterial growth and thus overcomes *xa5* resistance. However, *pthXo7* does not confer on PXO86 the same level of growth on *xa5* plants in comparison with PXO99^A, a strain compatible on *xa5*, nor do PthXo6-deficient mutants of PXO99^A suffer appreciable loss of virulence on plants with *xa5* (Sugio *et al.*, 2007). Therefore, PthXo7 does not appear to be the sole or even the principal reason for the ability of PXO99^A to cause significant disease in the presence of *xa5* (Sugio *et al.*, 2007). A third TAL effector has been identified recently in PXO99^A that provides a significant contribution to virulence and lesion formation, and preliminary findings have indicated that the effector targets aspects of host small RNA biosynthesis (B. Yang and F. F. White, unpublished results). Thus, *X. oryzae* pv. *oryzae* appears to have capitalized on the TAL effector family for multiple pathways to alter host physiology for the benefit of the pathogen.

TAL effector-mediated resistance has revealed novel defence adaptations in plant defence pathways. In addition to the recessive resistance afforded by *xa13*, which is a change in susceptibility, plants have exploited the function of TAL effectors by evolving promoters of genes that trigger a resistance response that contains TAL effector recognition features. In the case of the rice *R* gene *Xa27*, resistance is mediated by the elevated expression of *Xa27*, resulting in host cell death and resistance to disease (Gu *et al.*, 2005). *Xa27* induction is strictly dependent on a TAL effector named AvrXa27, the gene for which is present in many strains of *X. oryzae* pv. *oryzae* (Gu *et al.*, 2005). Susceptible rice cultivars have an identical ORF for *Xa27*, but the allele does not respond to AvrXa27-mediated induction, presumably as a result of sequence variations that are found in the promoter regions of the respective alleles. Similar to *Xa27*, the resistance in pepper caused by *Bs3*, the gene is induced in an AvrBs3-

dependent manner. Production of the Bs3 protein, which has structural features of a flavin oxidase, promotes the collapse and death of infected tissue (Romer *et al.*, 2007). The analysis of the Bs3 promoter revealed that, indeed, the apparent targets of AvrBs3 for virulence (*upa20* and possibly others) and *Bs3* share AvrBs3 binding sites (Romer *et al.*, 2009). *Xa27* and *Bs3* are not related on the basis of sequence comparison, and the question remains as to how many host proteins can function similarly to *Xa27* and *Bs3*. The rice *R* genes *Xa7* and *Xa10* are known to require intact TAL effector functions, and their analysis will provide more clues with regard to the diversity of host defence responses to this family of T3 effectors (Gu *et al.*, 2008; Porter *et al.*, 2003; Yang *et al.*, 2000; Zhu *et al.*, 1998).

At least one case, a TAL effector, triggers resistance in the presence of a better known type of *R* gene product. AvrBs4 (also known as AvrBsP and AvrBs3-2) is the cognate effector for the *R* gene *Bs4* (Schornack *et al.*, 2004). The Bs4 protein is a member of the Toll/interleukin 1 receptor-nucleotide binding site-leucine-rich repeat (TIR-NBS-LRR) class of R protein and is predicted to be a cytoplasmic protein. The question arises, therefore, as to whether the detection of AvrBs4 is related to its presence as a foreign protein within the host cell and unrelated to AvrBs4 function *per se*, or whether AvrBs4, in fact, has a non-nuclear function in the host (Gurlebeck *et al.*, 2009).

XopD

XopD has a small ubiquitin-like modifier (SUMO) protease domain, which is a member of the C48 protease family, and XopD has been shown to function *in vitro* in the release of SUMO from SUMO-modified plant proteins (Chosed *et al.*, 2007; Hotson *et al.*, 2003). XopD has also been shown to be nuclear localized (Hotson *et al.*, 2003) and, more recently, to have nonspecific DNA binding activity (Kim *et al.*, 2008). Mutagenesis results have indicated that the binding activity is located to a helix-loop-helix region spanning amino acids 113–131. One of the mutants (V118P) has been shown to reduce the ability of XopD to enhance virulence and delay the onset of necrosis at later infection times (12 days after inoculation), indicating that the DNA binding domain is required for maximal XopD effect. Two ethylene response factor-associated amphiphilic repression (EAR) motifs [L/FDLNL/F(x)P] were also detected within XopD at amino acids 244–249 and 284–289 (designated R1 and R2, respectively; Kim *et al.*, 2008). EAR motifs are found in plant transcription factors that repress defence and stress responses, presumably, as the exact modes of action are unknown, by binding activators of defence or developmental responses. Alterations to the EAR motifs resulted in a reduction in virulence and delayed necrosis effect of XopD, but did not affect the localization or catalytic activity of the proteins. Specific alterations to the catalytic site of XopD also resulted in a loss of virulence and

delayed necrosis (Kim *et al.*, 2008). In addition to the biological effects, XopD mutants have also been shown to result in the loss of defence gene expression in comparison with the suppression associated with XopD (Kim *et al.*, 2008). The SUMOylation of host proteins regulates protein stability and, as a consequence, processes in which the proteins are involved, including stress and developmental responses (Ulrich, 2009). Therefore, the hypothesis is that XopD may be directed to specific defence- or senescence-related transcription factors, either through DNA binding or the EAR motifs or both, and the catalytic activity of XopD destabilizes, by deSUMOylation, the factors involved in defence responses (Kim *et al.*, 2008).

XopJ

The XopJ group represents a subgroup of the large YopJ family of T3 effectors that are present in diverse pathogenic bacteria (Whalen *et al.*, 2008). These proteins are related to the C55 group of cysteine protease and, more specifically, have been proposed to be ubiquitin-like proteases and capable of removing SUMO proteins, which regulate the stability and activity of the modified (SUMOylated) host proteins. YopJ, itself, has been shown to have transacetylase activity and to acetylate serine and threonine residues of several mitogen-activated kinases (MAPs), preventing MAP kinase phosphorylation and signal transduction during innate immunity responses (Mukherjee *et al.*, 2006). The suppression of HR by AvrBsT in *Arabidopsis* by recessive mutation in *SOBER* has been cited as support for transacetylation in a plant system (Cunnac *et al.*, 2007). The results are consistent with a model whereby AvrBsT transacetylase activity is counteracted by the carboxylesterase activity of *SOBER* in most land races of *Arabidopsis* on an as yet unidentified host substrate. However, accession Pi-0 carries a null mutation in *SOBER* and undergoes an HR in an AvrBsT-dependent manner. The assumption is made that the carboxylesterase activity of *SOBER* would be hard to rationalize if AvrBsT was, in fact, a SUMO protease. Another member of the XopJ group, AvrRxv, induces an HR in the tomato cultivar Hawaii 7998, and the ability of AvrRxv to induce HR has been shown recently to require the conserved amino acid residues of the catalytic triad (Whalen *et al.*, 1993, 2008). Furthermore, the ability to elicit an HR was restored by the exchange of the catalytic domain of YopP, which is another member of the YopJ family from the animal pathogen *Vibrio parahaemolyticus* and has previously been shown to transacetylate MAP kinases, with the AvrRxv catalytic domain (Whalen *et al.*, 2008). Assuming that the N-terminal portion of AvrRxv does not alter the enzymatic function of the catalytic domain, and that the HR-eliciting activity is related to the virulence activity of AvrRxv, the results provide evidence for the transacetylase activity for AvrRxv. The requirement of AvrRxv for the conserved catalytic residues in the elicitation of HR is shared with AvrBsT (Orth *et al.*, 2000). Regardless,

different members of the XopJ group may function as transacetylases, and others, such as AvrXv4, may function as SUMO proteases (Roden *et al.*, 2004b).

The direct host targets of the members of the XopJ group are unknown for both resistance and virulence. XopJ has been shown to localize to the host cell membrane, and localization is at least partially dependent on the myristoylation motif at the N-terminus (Bartetzko *et al.*, 2009; Thieme *et al.*, 2007). XopJ has also been shown to inhibit host protein secretion and callose deposition, indicating possible interference with PTI signalling (Bartetzko *et al.*, 2009). AvrRxv binds a tomato 14-3-3 protein in the yeast two-hybrid assay (Whalen *et al.*, 2008). Binding is mediated by the N-terminal segment of AvrRxv (1–141 amino acids), which is proximal to the catalytic C-terminal portion. Binding has also been shown to be dependent on an apparent 14-3-3 binding motif, known as the R18 domain, which is present in AvrRxv. Amino acid substitutions that disrupt the R18 domain also result in the loss of HR elicitation by AvrRxv on delivery by *Agrobacterium*-mediated gene transfer (Whalen *et al.*, 2008). 14-3-3 proteins function as adapter proteins in a variety of cellular complexes, including MAP kinase pathways, and AvrRxv may be, in part, localized to the site of action by binding to a specific 14-3-3 protein involved in host innate immunity signalling, possibly the MAP kinase pathway (Whalen *et al.*, 2008). Once localized, AvrRxv would then transacetylate the as yet unknown host protein and prevent signal transduction.

XopN

Using a yeast two-hybrid screen, XopN has been shown to interact with a variety of proteins from tomato, including an atypical (phosphorylation-deficient) receptor-link kinase named TARK1 and a 14-3-3 protein named TFT1. TARK1, similar to other RLK proteins, spans the cell membrane with the LRR domain external to the cell and the kinase (in this case, nonfunctional) domain in the cytoplasm. The interaction with TARK1 has been shown to require a LXXLL sequence in the cytoplasmic portion of TARK1. Alteration of the LXXLL motif resulted in a loss of TARK1 binding to XopN and binding was shown to require a LXXLL motif in the N-terminal portion of XopN. A nonbinding mutant of XopN was then constructed and tested for effects on the biological phenotypes caused by XopN in *X. campestris* pv. *vesicatoria*. The loss of TARK1 binding resulted in an approximately 50% reduction in the virulence effect of wild-type XopN and did not affect the suppression of callose deposition (Kim *et al.*, 2009). The effect on suppression of defence-related gene expression was not reported. Therefore, some evidence supports the fact that the interaction of TARK1 and XopN controls, at least in part, the effects of XopN in disease. Furthermore, suppression of TARK1 expression in tomato also resulted in partial suppression of

XopN-mediated susceptibility (Kim *et al.*, 2009). Bacteria without XopN grew to higher populations in plants with reduced TARK1 expression when compared with growth in plants with a wild-type TARK1. TARK1-suppressed plants also displayed reduced disease symptoms, presenting the possibility that TARK1, indeed, may be involved in host defence responses (Kim *et al.*, 2009). Atypical kinases have no intrinsic kinase activity and presumably mediate their effects solely through interactions with other components of the RLK pathways (Castells and Casacuberta, 2007). The involvement of the 14-3-3 proteins, which are known to be involved in signal transduction in plants and animals, with XopN remains to be shown. XopN contains several anti-parallel α -helical repeats, or HEAT (huntingtin, elongation factor 3, PR65/A, TOR1) repeats, the function of which is unknown at present (Roden *et al.*, 2004a). The repeats of XopN may be involved in interactions with TFT1 or other host proteins.

XopAC and XopAH

Two T3 effectors of *Xanthomonas* contain sequences related to the recently described Fido domain, which is derived from the fic [cyclic adenosine monophosphate (cAMP)-induced filamentation] and doc (death on curing) domains (Kinch *et al.*, 2009). Structural comparisons resulted in the inclusion of similar segments of the T3 effector AvrB from *P. syringae* species (Kinch *et al.*, 2009). A fido domain is present in the T3 effector from the animal pathogen *Vibrio parahaemolyticus*, named VopS, which causes host cell cytotoxicity and covalently modifies Rho GTPases with adenosine monophosphate moieties. The new modification has been called an AMPylation reaction and has been proposed to alter signalling functions of the target proteins, similar to phosphorylation, ribosylation and acetylation modifications (Yarbrough and Orth, 2009). The family contains central motif sequences (HPFx[D/E]GN[G/K]R), with histidine contributing to AMPylation. Although AvrB and related proteins lack the histidine residue, other residues within the structurally related region have been proposed to serve the same function (Kinch *et al.*, 2009). XopAC (XCC2565) of *X. campestris* pv. *campestris* contains the consensus fic domain, whereas XopAH (XCC2109) from *X. campestris* pv. *campestris* has sequence similarity to AvrB (Table 1). Presumably, T3 effectors in the XopAC and XopAH groups could trans-AMPylation plant host proteins, as shown for VopS. Interestingly, XopAH from *X. campestris* pv. *campestris* strain 8004 also contains an identical ORF (XC_1553) to XCC2565 of strain ATCC33913, which was named AvrAC (Xu *et al.*, 2008). Although AvrAC has been shown not to modulate virulence in susceptible plants, the effector elicits a resistance reaction in landrace Col-0 of *Arabidopsis*. Curiously, resistance appears to be limited to the vascular system, implicating tissue specificity for resistance and possibly the virulence functions of T3 effectors (Xu *et al.*, 2008).

Table 4 Cloned *R* genes for resistance to *Xanthomonas* species.

Gene	Class (plant)	Comments*	Cognate T3 effector	Reference
<i>Bs2</i>	NBS-LRR (pepper)		AvrBs2	Tai <i>et al.</i> , 1999
<i>Bs3</i>	Inducible (pepper)	Flavin oxygenase-related protein	AvrBs3	Romer <i>et al.</i> , 2007
<i>Bs3-E</i>	Inducible (pepper)	Promoter deletion of <i>Bs3</i>	AvrBs3Δ16	Romer <i>et al.</i> , 2009
<i>Bs4</i>	NBS-LRR (tomato)	Cytoplasm	AvrBs4 (AvrBsP; AvrBs3-2)	Schornack <i>et al.</i> , 2004
<i>Rxo1</i>	NBS-LRR (maize)	Cytoplasm; broad resistance; transferred to rice from maize	AvrXo1	Zhao <i>et al.</i> , 2005
<i>Xa1</i>	NBS-LRR (rice)	Cytoplasm	Unknown	Yoshimura <i>et al.</i> , 1998
<i>xa13</i>	Promoter mutants of <i>Os8N3</i> ; nodulin 3 family (rice)	Membrane protein; unresponsive <i>S</i> gene to TAL effector PthXo1	PthXo1	Chu <i>et al.</i> , 2006; Yang <i>et al.</i> , 2006
<i>Xa21</i>	RLK (rice)	Extracellular receptor, broad resistance	None	Song <i>et al.</i> , 1995
<i>Xa26/Xa3</i>	RLK (rice)	Extracellular receptor, broad resistance	None	Xiang <i>et al.</i> , 2006
<i>Xa27</i>	Inducible (rice)	Membrane and cell wall; novel protein; broad resistance	AvrXa27	Gu <i>et al.</i> , 2005
<i>xa5</i>	Missense mutant of TFIIAγ5; small subunit of TFIIA transcription factor complex (rice)	Nuclear; broad resistance	Unknown	Jiang <i>et al.</i> , 2006; Iyer and McCouch, 2004

*Comments in relation to *R* gene product localization, resistance profile, putative function/structure.

COGNATE *R* GENES TO T3 EFFECTORS OF *XANTHOMONAS*

A greater goal for understanding T3 effector function is to place it in the context of host/pathogen adaptation and to determine whether the knowledge can assist in the design or selection of durable and broad resistance. Host adaptation to disease involves the evolution of modified defence surveillance mechanisms in response to the adaptive processes for virulence within pathogen populations. A variety of *R* genes that are directed against *Xanthomonas* species have been cloned and characterized (Table 4). The two largest and best-characterized classes of protein that function as *R* genes are the aforementioned RLKs and NBS-LRR, and, perhaps not surprisingly, over one-half of the genes are members of these two classes. *Bs2*, *Rxo1*, *Xa1* and *Bs4* encode members of the NBS-LRR family. *Xa21* and *Xa26*, as members of the RLK class, represent components of the PRR line of defence in rice and, in themselves, are not cognate *R* genes corresponding to specific T3 effectors. However, the case for suppression of flagellin/FLS2-mediated PRR signalling by T3 effectors is well advanced in the studies of *P. syringae*, and interesting questions arise regarding T3 effectors with related functions from *Xanthomonas* (Boller and He, 2009). One can anticipate the identification of specific T3 effectors in *Xanthomonas* directed against this class of protein. If species of *Xanthomonas*, in general, are proficient in RLK signal transduction suppression, strains may adapt to the presence of *Xa21*, *Xa26* or similar *R* genes through the acquisition of PRR pathway suppressors.

In addition to major classes of *R* genes, studies of *Xanthomonas* diseases of rice have resulted in the identification of a variety of novel additions to the *R* gene pantheon, indicating that the struggle continues within the host nucleus (White and Yang, 2009). The host transcriptional machinery is under attack, as revealed by studies of XopD and the various TAL effectors. *Xa27*

and *Bs3* reflect newly discovered strategies to abrogate infection. The induction of self-inflicted cell damage, as mediated by TAL effector recognition, seems to be remarkably analogous to the phenomenon of host lesion mimics. The host is under apparent selective pressure to evolve toxic gene–promoter combinations that react to TAL effectors. *Xa27* and *Bs3* products are not related on the basis of sequence or, seemingly, biochemical function. *Xa27* is targeted to the host membrane, whereas *Bs3* appears to represent a cytoplasmic protein. It will be interesting to discover how many other host proteins exist that can potentially trigger a defensive reaction on expression. The two recessive resistance genes, *xa5* and *xa13*, directed at *X. oryzae* pv. *oryzae*, also represent new insights into host adaptations for resistance and will undoubtedly also provide insight into TAL effector function (Chu *et al.*, 2006; Iyer and McCouch, 2004; Jiang *et al.*, 2006; Yang *et al.*, 2006). Both are hypothesized to interfere with effector function, albeit by different mechanisms. *xa5*, as a component of the transcription complex, may function by interference with general TAL effector interaction with the host transcription machinery. *xa13*, by contrast, interferes with the action of a specific TAL effector, namely PthXo1 (Yang *et al.*, 2006).

CONCLUSIONS

The experimental analyses of individual T3 effectors of *Xanthomonas* have provided a unique view of the interactions of plant pathogenic bacteria and their hosts, and genomic sequence analysis has provided an assessment of the similarities with related pathogens and the respective armamentarium of T3 effectors. At the same time, advances in sequencing technology also promise to provide unprecedented access to the diversity of T3 effectors in *Xanthomonas* as well as other species. Some insights into the aspects of host/pathogen adaptation are already apparent from the recent findings for the TAL effector family. Results

with XopJ, AvrRxv, XopD and XopN also implicate a complexity of interactions with host factors. Structural studies of T3 effectors may yield functional similarities not detectable by primary sequence comparisons. Continued advancements in the examination of physiological effects of individual proteins, such as protoplast systems, *Agrobacterium*-mediated transfer and T3 effector gene-deficient strains of the pathogens, will assist in the identification of the individual contributions of each candidate. In some respect, examination of the different effector families is a view of plant–pathogen interactions backwards through time. Groups with highly diverse members shared among plant and animal effectors may reflect the evolutionarily conserved defence pathways of plants and animals. Other families, including the AvrBs3 family, may be newly emerging virulence factors, and have only recently spread throughout the genus. Undoubtedly, the groupings of Xop will be revised, and manual revisions in T3 effector annotation will be required. Additional improvements in T3 effector validation on a genomic scale will also facilitate research. Nevertheless, a plethora of virtually unexamined T3 effectors exist, and the T3 effectors of *Xanthomonas* will provide fuel for many more studies and insights in the next few years.

REFERENCES

- Almeida, N.F., Yan, S., Lindeberg, M., Studholme, D.J., Schneider, D.J., Condon, B., Liu, H., Viana, C.J., Warren, A., Evans, C., Kemen, E., Maclean, D., Angot, A., Martin, G.B., Jones, J.D., Collmer, A., Setubal, J.C. and Vinatzer, B.A. (2009) A draft genome sequence of *Pseudomonas syringae* pv. *tomato* T1 reveals a type III effector repertoire significantly divergent from that of *Pseudomonas syringae* pv. *tomato* DC3000. *Mol. Plant–Microbe Interact.* **22**, 52–62.
- Al-Saadi, A., Reddy, J.D., Duan, Y.P., Brunings, A.M., Yuan, Q. and Gabriel, D.W. (2007) All five host-range variants of *Xanthomonas citri* carry one *pthA* homolog with 17.5 repeats that determines pathogenicity on citrus, but none determine host-range variation. *Mol. Plant–Microbe Interact.* **20**, 934–943.
- Aritua, V., Parkinson, N., Thwaites, R., Heeney, J.V., Jones, D.R., Tushemereirwe, W., Crozier, R., Reeder, R., Stead, D. and Smith, J. (2008) Characterization of the *Xanthomonas* sp. causing wilt of onset and banana and its proposed reclassification as a strain of *X. vasicola*. *Plant Pathol.* **57**, 170–177.
- Astua-Monge, G., Minsavage, G.V., Stall, R.E., Davis, M.J., Bonas, U. and Jones, J.B. (2000a) Resistance of tomato and pepper to T3 strains of *Xanthomonas campestris* pv. *vesicatoria* is specified by a plant-inducible avirulence gene. *Mol. Plant–Microbe Interact.* **13**, 911–921.
- Astua-Monge, G., Minsavage, G.V., Stall, R.E., Vallejos, C.E., Davis, M.J. and Jones, J.B. (2000b) *Xv4-avrXv4*: a new gene-for-gene interaction identified between *Xanthomonas campestris* pv. *vesicatoria* race T3 and wild tomato relative *Lycopersicon pennellii*. *Mol. Plant–Microbe Interact.* **13**, 1346–1355.
- Bai, J., Choi, S.H., Ponciano, G., Leung, H. and Leach, J.E. (2000) *Xanthomonas oryzae* pv. *oryzae* avirulence genes contribute differently and specifically to pathogen aggressiveness. *Mol. Plant–Microbe Interact.* **13**, 1322–1329.
- Bartetzko, V., Sonnewald, S., Vogel, F., Hartner, K., Stadler, R., Hammes, U.Z. and Bornke, F. (2009) The *Xanthomonas campestris* pv. *vesicatoria* type III effector protein XopJ inhibits protein secretion: evidence for interference with cell wall-associated defense responses. *Mol. Plant–Microbe Interact.* **22**, 655–664.
- Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406.
- Boller, T. and He, S.Y. (2009) Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, **324**, 742–744.
- Bonas, U., Conrads-Strauch, J. and Balbo, I. (1993) Resistance in tomato to *Xanthomonas campestris* pv. *vesicatoria* is determined by alleles of the pepper-specific avirulence gene *avrBs3*. *Mol. Gen. Genet.* **238**, 261–269.
- Bonas, U., Stall, R.E. and Staskawicz, B.J. (1989) Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Gen. Genet.* **218**, 127–136.
- Bretz, J.R., Mock, N.M., Charity, J.C., Zeyad, S., Baker, C.J. and Hutcheson, S.W. (2003) A translocated protein tyrosine phosphatase of *Pseudomonas syringae* pv. *tomato* DC3000 modulates plant defence response to infection. *Mol. Microbiol.* **49**, 389–400.
- Brown, I., Mansfield, J. and Bonas, U. (1995) *hrp* genes in *Xanthomonas campestris* pv. *vesicatoria* determine ability to suppress papilla deposition in pepper mesophyll cells. *Mol. Plant–Microbe Interact.* **8**, 825–836.
- Buttner, D. and He, S.Y. (2009) Type III protein secretion in plant pathogenic bacteria. *Plant Physiol.* **150**, 1656–1664.
- Casper-Lindley, C., Dahlbeck, D., Clark, E.T. and Staskawicz, B.J. (2002) Direct biochemical evidence for type III secretion-dependent translocation of the AvrBs2 effector protein into plant cells. *Proc. Natl. Acad. Sci. USA*, **99**, 8336–8341.
- Castaneda, A., Reddy, J.D., El-Yacoubi, B. and Gabriel, D.W. (2005) Mutagenesis of all eight *avr* genes in *Xanthomonas campestris* pv. *campestris* had no detected effect on pathogenicity, but one *avr* gene affected race specificity. *Mol. Plant–Microbe Interact.* **18**, 1306–1317.
- Castells, E. and Casacuberta, J.M. (2007) Signaling through kinase-defective domains: the prevalence of atypical receptor-like kinases in plants. *J. Exp. Bot.* **58**, 3503–3511.
- Chakrabarty, P.K., Duan, Y.P. and Gabriel, D.W. (1997) Cloning and characterization of a member of the *Xanthomonas avr/pth* gene family that evades all commercially utilized cotton R genes in the United States. *Phytopathology*, **87**, 1160–1167.
- Chosed, R., Tomchick, D.R., Brautigam, C.A., Mukherjee, S., Negi, V.S., Machius, M. and Orth, K. (2007) Structural analysis of *Xanthomonas* XopD provides insights into substrate specificity of ubiquitin-like protein proteases. *J. Biol. Chem.* **282**, 6773–6782.
- Chu, Z., Yuan, M., Yao, J., Ge, X., Yuan, B., Xu, C., Li, X., Fu, B., Li, Z., Bennetzen, J.L., Zhang, Q. and Wang, S. (2006) Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes. Dev.* **20**, 1250–1255.
- Ciesiolka, L.D., Hwin, T., Gearlds, J.D., Minsavage, G.V., Saenz, R., Bravo, M., Handley, V., Conover, S.M., Zhang, H., Caporgno, J., Phengrasamy, N.B., Toms, A.O., Stall, R.E. and Whalen, M.C. (1999) Regulation of expression of avirulence gene *avrRxv* and identification of a family of host interaction factors by sequence analysis of *avrBsT*. *Mol. Plant–Microbe Interact.* **12**, 35–44.
- Collmer, A., Schneider, D.J. and Lindeberg, M. (2009) Lifestyles of the

- effector-rich: genome-enabled characterization of bacterial plant pathogens. *Plant Physiol.* **150**, 1623–1630.
- Cunnac, S., Lindeberg, M. and Collmer, A. (2009) *Pseudomonas syringae* type III secretion system effectors: repertoires in search of functions. *Curr. Opin. Microbiol.* **12**, 53–60.
- Cunnac, S., Wilson, A., Nuwer, J., Kirik, A., Baranage, G. and Mudgett, M.B. (2007) A conserved carboxylesterase is a SUPPRESSOR OF AVRBS1-ELICITED RESISTANCE in Arabidopsis. *Plant Cell*, **19**, 688–705.
- De Feyter, R., Yang, Y. and Gabriel, D.W. (1993) Gene-for-genes interactions between cotton R genes and *Xanthomonas campestris* pv. *malvacearum* avr genes. *Mol. Plant–Microbe Interact.* **6**, 225–237.
- Duan, Y.P., Castañeda, A., Zhao, G., Erdos, G. and Gabriel, D.W. (1999) Expression of a single, host-specific, bacterial pathogenicity gene in plant cells elicits division, enlargement, and cell death. *Mol. Plant–Microbe Interact.* **12**, 556–560.
- Erbs, G., Silipo, A., Aslam, S., De Castro, C., Liparoti, V., Flagiello, A., Pucci, P., Lanzetta, R., Parrilli, M., Molinaro, A., Newman, M.A. and Cooper, R.M. (2008) Peptidoglycan and muropeptides from pathogens *Agrobacterium* and *Xanthomonas* elicit plant innate immunity: structure and activity. *Chem. Biol.* **15**, 438–448.
- Espinosa, A., Guo, M., Tam, V.C., Fu, Z.Q. and Alfano, J.R. (2003) The *Pseudomonas syringae* type III-secreted protein HopPtoD2 possesses protein tyrosine phosphatase activity and suppresses programmed cell death in plants. *Mol. Microbiol.* **49**, 377–387.
- Feil, H., Feil, W.S., Chain, P., Larimer, F., DiBartolo, G., Copeland, A., Lykidis, A., Trong, S., Nolan, M., Goltsman, E., Thiel, J., Malfatti, S., Loper, J.E., Lapidus, A., Detter, J.C., Land, M., Richardson, P.M., Kypides, N.C., Ivanova, N. and Lindow, S.E. (2005) Comparison of the complete genome sequences of *Pseudomonas syringae* pv. *syringae* B728a and pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. USA*, **102**, 11064–11069.
- Furutani, A., Takaoka, M., Sanada, H., Noguchi, Y., Oku, T., Tsuno, K., Ochiai, H. and Tsuge, S. (2009) Identification of novel type III secretion effectors in *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant–Microbe Interact.* **22**, 96–106.
- Gassmann, W., Dahlbeck, D., Chesnokova, O., Minsavage, G.V., Jones, J.B. and Staskawicz, B.J. (2000) Molecular evolution of virulence in natural field strains of *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.* **182**, 7053–7059.
- Gonzalez, C., Szurek, B., Manceau, C., Mathieu, T., Séré, Y. and Verdier, V. (2007) Molecular and pathotypic characterization of new *Xanthomonas oryzae* strains from West Africa. *Mol. Plant–Microbe Interact.* **20**, 534–546.
- Greenberg, J.T. and Vinatzer, B.A. (2003) Identifying type III effectors of plant pathogens and analyzing their interaction with plant cells. *Curr. Opin. Microbiol.* **6**, 20–28.
- Gu, K., Sangha, J.S., Li, Y. and Yin, Z. (2008) High-resolution genetic mapping of bacterial blight resistance gene *Xa10*. *Theor. Appl. Genet.* **116**, 155–163.
- Gu, K., Tian, D., Yang, F., Wu, L., Sreekala, C., Wang, D., Wang, G.L. and Yin, Z. (2004) High-resolution genetic mapping of *Xa27(t)*, a new bacterial blight resistance gene in rice, *Oryza sativa* L. *Theor. Appl. Genet.* **108**, 800–807.
- Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G.L., White, F.F. and Yin, Z. (2005) R gene expression induced by a type-III effector triggers disease resistance in rice. *Nature*, **435**, 1122–1125.
- Gurlebeck, D., Jahn, S., Gurlebeck, N., Szczesny, R., Szurek, B., Hahn, S., Hause, G. and Bonas, U. (2009) Visualization of novel virulence activities of *Xanthomonas* type III effectors AvrBs1, AvrBs3, and AvrBs4. *Mol. Plant Pathol.* **10**, 175–188.
- Guttman, D.S., Vinatzer, B.A., Sarkar, S.F., Ranall, M.V., Kettler, G. and Greenberg, J.T. (2002) A functional screen for the type III (Hrp) secretome of the plant pathogen *Pseudomonas syringae*. *Science*, **295**, 1722–1726.
- Han, S., Arvai, A.S., Clancy, S.B. and Tainer, J.A. (2001) Crystal structure and novel recognition motif of rho ADP-ribosylating C3 exoenzyme from *Clostridium botulinum*: structural insights for recognition specificity and catalysis. *J. Mol. Biol.* **305**, 95–107.
- Han, S. and Tainer, J.A. (2002) The ARTT motif and a unified structural understanding of substrate recognition in ADP-ribosylating bacterial toxins and eukaryotic ADP-ribosyltransferases. *Int. J. Med. Microbiol.* **291**, 523–529.
- Hayward, A.C. (1993) The hosts of *Xanthomonas*. In: *Xanthomonas* (Swings, J.G. and Civerolo, E.L., eds), pp. 1–17. London: Chapman and Hall.
- Herbers, K., Conrads-Strauch, J. and Bonas, U. (1992) Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. *Nature*, **356**, 172–174.
- Hogenhout, S.A., Van der Hoorn, R.A., Rerauchi, R. and Kamoun, S. (2009) Emerging concepts in effector biology of plant-associated organisms. *Mol. Plant–Microbe Interact.* **22**, 115–122.
- Hopkins, C.M., White, F.F., Choi, S., Guo, A. and Leach, J.E. (1992) Identification of a family of avirulence genes from *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant–Microbe Interact.* **5**, 451–459.
- Hotson, A., Chosed, R., Shu, H., Orth, K. and Mudgett, M.B. (2003) *Xanthomonas* type III effector XopD targets SUMO-conjugated proteins in planta. *Mol. Microbiol.* **50**, 377–389.
- Hotson, A. and Mudgett, M.B. (2004) Cysteine proteases in phytopathogenic bacteria: identification of plant targets and activation of innate immunity. *Curr. Opin. Plant Biol.* **7**, 384–390.
- Iyer, A.S. and McCouch, S.R. (2004) The rice bacterial blight resistance gene *xa5* encodes a novel form of disease resistance. *Mol. Plant–Microbe Interact.* **17**, 1348–1354.
- Jha, G., Rajeshwari, R. and Sonti, R.V. (2007) Functional interplay between two *Xanthomonas oryzae* pv. *oryzae* secretion systems in modulating virulence on rice. *Mol. Plant–Microbe Interact.* **20**, 31–40.
- Jiang, B.L., He, Y.Q., Cen, W.J., Wei, H.Y., Jiang, G.F., Jiang, W., Hang, X.H., Feng, J.X., Lu, G.T., Tang, D.J. and Tang, J.L. (2008) The type III secretion effector XopXccN of *Xanthomonas campestris* pv. *campestris* is required for full virulence. *Res. Microbiol.* **159**, 216–220.
- Jiang, G.H., Xia, Z.H., Zhou, Y.L., Wan, J., Li, D.Y., Chen, R.S., Zhai, W.X. and Zhu, L.H. (2006) Testifying the rice bacterial blight resistance gene *xa5* by genetic complementation and further analyzing *xa5* (*Xa5*) in comparison with its homolog *TFIIAγ1*. *Mol. Genet. Genomics*, **275**, 354–366.
- Jones, J.B., Bouzar, H., Stall, R.E., Almira, E.C., Roberts, P.D., Bowen, B.W., Sudberry, J., Strickler, P.M. and Chun, J. (2000) Systematic analysis of xanthomonads (*Xanthomonas* spp.) associated with pepper and tomato lesions. *Int. J. Syst. Evol. Microbiol.* **50**, Pt 3: 1211–1219.
- Kanamori, H. and Tsuyumu, S. (1998) Comparison of nucleotide sequences of canker-forming and non-canker-forming *pthA* homologues in *Xanthomonas campestris* pv. *citri*. *Ann. Phytopathol. Soc. Jpn.* **64**, 462–470.

- Kay, S., Boch, J. and Bonas, U. (2005) Characterization of AvrBs3-like effectors from a Brassicaceae pathogen reveals virulence and avirulence activities and a protein with a novel repeat architecture. *Mol. Plant-Microbe Interact.* **18**, 838–848.
- Kay, S., Hahn, S., Marois, E., Hause, G. and Bonas, U. (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science*, **318**, 648–651.
- Kay, S., Hahn, S., Marois, E., Wieduwild, R. and Bonas, U. (2009) Detailed analysis of the DNA recognition motifs of the *Xanthomonas* type III effectors AvrBs3 and AvrBs3delta16. *Plant J.* **59**, 859–871.
- Kearney, B. and Staskawicz, B.J. (1990) Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. *Nature*, **346**, 385–386.
- Keshavarzi, M., Soylu, S., Brown, I., Bonas, U., Nicole, M., Rossiter, J. and Mansfield, J. (2004) Basal defenses induced in pepper by lipopolysaccharides are suppressed by *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Plant-Microbe Interact.* **17**, 805–815.
- Kim, J.G., Li, X., Roden, J.A., Taylor, K.W., Aakre, C.D., Su, B., Lalonde, S., Kirik, A., Chen, Y., Baranage, G., McLane, H., Martin, G.B. and Mudgett, M.B. (2009) *Xanthomonas* T3S effector XopN suppresses PAMP-triggered immunity and interacts with a tomato atypical receptor-like kinase and TFT1. *Plant Cell*, **21**, 1305–1323.
- Kim, J.G., Park, B.K., Yoo, C.H., Jeon, E., Oh, J. and Hwang, I. (2003) Characterization of the *Xanthomonas axonopodis* pv. *glycines* Hrp pathogenicity island. *J. Bacteriol.* **185**, 3155–3166.
- Kim, J.G., Taylor, K.W., Hotson, A., Keegan, M., Schmelz, E.A. and Mudgett, M.B. (2008) XopD SUMO protease affects host transcription, promotes pathogen growth, and delays symptom development in *Xanthomonas*-infected tomato leaves. *Plant Cell*, **20**, 1915–1929.
- Kinch, L.N., Yarbrough, M.L., Orth, K. and Grishin, N.V. (2009) Fido, a novel AMPylation domain common to Fic, Doc, and AvrB. *PLoS ONE*, **4**, e5818.
- Lalli, G., Bohnert, S., Deinhardt, K., Verastegui, C. and Schiavo, G. (2003) The journey of tetanus and botulinum neurotoxins in neurons. *Trends Microbiol.* **11**, 431–437.
- Lee, B.M., Park, Y.J., Park, D.S., Kang, H.W., Kim, J.G., Song, E.S., Park, I.C., Yoon, U.H., Hahn, J.H., Koo, B.S., Lee, G.B., Kim, H., Park, H.S., Yoon, K.O., Kim, J.H., Jung, C.H., Koh, N.H., Seo, J.S. and Go, S.J. (2005) The genome sequence of *Xanthomonas oryzae* pathovar *oryzae* KACC10331, the bacterial blight pathogen of rice. *Nucleic Acids Res.* **33**, 577–586.
- Lee, K.S., Rasabandith, S., Angeles, E.R. and Khush, G.S. (2003) Inheritance of resistance to bacterial blight in 21 cultivars of rice. *Phytopathology*, **93**, 147–152.
- Lee, S.W., Han, S.W., Bartley, L.E. and Ronald, P.C. (2006) From the academy: colloquium review. Unique characteristics of *Xanthomonas oryzae* pv. *oryzae* AvrXa21 and implications for plant innate immunity. *Proc. Natl. Acad. Sci. USA*, **103**, 18395–18400.
- Leyns, F., De Cleene, M., Swings, J.G. and De Ley, J. (1984) The host range of the genus *Xanthomona*. *Bot. Rev.* **50**, 308–356.
- Lindeberg, M., Biehl, B.S., Glasner, J.D., Perna, N.T., Collmer, A. and Collmer, C.W. (2009) Gene ontology annotation highlights shared and divergent pathogenic strategies of type III effector proteins deployed by the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 and animal pathogenic *Escherichia coli* strains. *BMC Microbiol.* **9** (Suppl. 1), S4.
- Marois, E., van den Ackerveken, G. and Bonas, U. (2002) The *Xanthomonas* type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host. *Mol. Plant-Microbe Interact.* **15**, 637–646.
- Metz, M., Dahlbeck, D., Morales, C.Q., Al Sady, B., Clark, E.T. and Staskawicz, B.J. (2005) The conserved *Xanthomonas campestris* pv. *vesicatoria* effector protein XopX is a virulence factor and suppresses host defense in *Nicotiana benthamiana*. *Plant J.* **41**, 801–814.
- Morales, C.Q., Posada, J., Macneale, E., Franklin, D., Rivas, I., Bravo, M., Minsavage, J., Stall, R.E. and Whalen, M.C. (2005) Functional analysis of the early chlorosis factor gene. *Mol. Plant-Microbe Interact.* **18**, 477–486.
- Mukaihara, T. and Tamura, N. (2009) Identification of novel *Ralstonia solanacearum* type III effector proteins through translocation analysis of hrpB-regulated gene products. *Microbiology*, **155**, 2235–2244.
- Mukherjee, S., Hao, Y.H. and Orth, K. (2007) A newly discovered post-translational modification—the acetylation of serine and threonine residues. *Trends Biochem. Sci.* **32**, 210–216.
- Mukherjee, S., Keitany, G., Li, Y., Wang, Y., Ball, H.L., Goldsmith, E.J. and Orth, K. (2006) *Yersinia* YopJ acetylates and inhibits kinase activation by blocking phosphorylation. *Science*, **312**, 1211–1214.
- Nimchuk, Z.L., Fisher, E.J., Desveaux, D., Chang, J.H. and Dangl, J.L. (2007) The HopX (AvrPphE) family of *Pseudomonas syringae* type III effectors requires a catalytic triad and a novel N-terminal domain for function. *Mol. Plant-Microbe Interact.* **20**, 346–357.
- Nimchuk, Z., Marois, E., Kjemtrup, S., Leister, R.T., Katagiri, F. and Dangl, J.L. (2000) Eukaryotic fatty acylation drives plasma membrane targeting and enhances function of several type III effector proteins from *Pseudomonas syringae*. *Cell*, **101**, 353–363.
- Noel, L., Thieme, F., Gabler, J., Buttner, D. and Bonas, U. (2003) XopC and XopJ, two novel type III effector proteins from *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.* **185**, 7092–7102.
- Noel, L., Thieme, F., Nennstiel, D. and Bonas, U. (2002) Two novel type III-secreted proteins of *Xanthomonas campestris* pv. *vesicatoria* are encoded within the hrp pathogenicity island. *J. Bacteriol.* **184**, 1340–1348.
- Ochiai, H., Inoue, Y., Takeya, M., Sasaki, A. and Kaku, H. (2005) Genome sequence of *Xanthomonas oryzae* pv. *oryzae* suggests contribution of large numbers of effector genes and insertion sequences to its race diversity. *JARQ*, **39**, 275–287.
- Orth, K., Xu, Z., Mudgett, M.B., Bao, Z.Q., Palmer, L.E., Bliska, J.B., Mangel, W.F., Staskawicz, B. and Dixon, J.E. (2000) Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease. *Science*, **290**, 1594–1597.
- Porter, B., Chittoor, J., Yano, M., Sasaki, T. and White, F.F. (2003) Development and mapping of markers linked to the rice bacterial blight resistance gene *Xa7*. *Crop. Sci.* **43**, 1484–1492.
- Qian, W., Jia, Y., Ren, S.X., He, Y.Q., Feng, J.X., Lu, L.F., Sun, Q., Ying, G., Tang, D.J., Tang, H., Wu, W., Hao, P., Wang, L., Jiang, B.L., Zeng, S., Gu, W.Y., Lu, G., Rong, L., Tian, Y., Yao, Z., Fu, G., Chen, B., Fang, R., Qiang, B., Chen, Z., Zhao, G.P., Tang, J.L. and He, C. (2005) Comparative and functional genomic analyses of the pathogenicity of phytopathogen *Xanthomonas campestris* pv. *campestris*. *Genome. Res.* **15**, 757–767.

- Roden, J.A., Belt, B., Ross, J.B., Tachibana, T., Vargas, J. and Mudgett, M.B. (2004a) A genetic screen to isolate type III effectors translocated into pepper cells during *Xanthomonas* infection. *Proc. Natl. Acad. Sci. USA*, **101**, 16624–16629.
- Roden, J., Eardley, L., Hotson, A., Cao, Y. and Mudgett, M.B. (2004b) Characterization of the *Xanthomonas* AvrXv4 effector, a SUMO protease translocated into plant cells. *Mol. Plant–Microbe Interact.* **17**, 633–643.
- Romer, P., Hahn, S., Jordan, T., Strauss, T., Bonas, U. and Lahaye, T. (2007) Plant pathogen recognition mediated by promoter activation of the pepper *Bs3* resistance gene. *Science*, **318**, 645–648.
- Romer, P., Strauss, T., Hahn, S., Scholze, H., Morbitzer, R., Grau, J., Bonas, U. and Lahaye, T. (2009) Recognition of AvrBs3-like proteins is mediated by specific binding to promoters of matching pepper *Bs3* alleles. *Plant Physiol.* **150**, 1677–1686.
- Ronald, P.C. and Stasiewicz, B.J. (1988) The avirulence gene *avrBs1* from *Xanthomonas campestris* pv. *vesicatoria* encodes a 50-kD protein. *Mol. Plant–Microbe Interact.* **1**, 191–198.
- Rosebrock, T.R., Zeng, L., Brady, J.J., Abramovitch, R.B., Xiao, F. and Martin, G.B. (2007) A bacterial E3 ubiquitin ligase targets a host protein kinase to disrupt plant immunity. *Nature*, **448**, 370–374.
- Rybak, M., Minsavage, G.V., Stall, R.E. and Jones, J.B. (2009) Identification of *Xanthomonas citri* ssp. *citri* host specificity genes in a heterologous expression host. *Mol. Plant Pathol.* **10**, 249–262.
- Salanoubat, M., Genin, S., Artiguenave, F., Gouzy, J., Mangenot, S., Arlat, M., Billault, A., Brottier, P., Camus, J.C., Cattolico, L., Chandler, M., Choise, N., Claudel-Renard, C., Cunac, S., Demange, N., Gaspin, C., Lavie, M., Moisan, A., Robert, C., Saurin, W., Schiex, T., Siguier, P., Thebault, P., Whalen, M., Wincker, P., Levy, M., Weissenbach, J. and Boucher, C.A. (2002) Genome sequence of the plant pathogen *Ralstonia solanacearum*. *Nature*, **415**, 497–502.
- Salzberg, S.L., Sommer, D.D., Schatz, M.C., Phillippy, A.M., Rabinowicz, P.D., Tsuge, S., Furutani, A., Ochiai, H., Delcher, A.L., Kelley, D., Madupu, R., Puiu, D., Radune, D., Shumway, M., Trapnell, C., Aparna, G., Jha, G., Pandey, A., Patil, P.B., Ishihara, H., Meyer, D.F., Szurek, B., Verdier, V., Koebnik, R., Dow, J.M., Ryan, R.P., Hirata, H., Tsuyumu, S., Won Lee, S., Ronald, P.C., Sonti, R.V., Van Sluys, M.A., Leach, J.E., White, F.F. and Bogdanove, A.J. (2008) Genome sequence and rapid evolution of the rice pathogen *Xanthomonas oryzae* pv. *oryzae* PXO99^A. *BMC Genomics*, **9**, 204.
- Schechter, L.M., Roberts, K.A., Jamir, Y., Alfano, J.R. and Collmer, A. (2004) *Pseudomonas syringae* type III secretion system targeting signals and novel effectors studied with a *Cya* translocation reporter. *J. Bacteriol.* **186**, 543–555.
- Schornack, S., Ballvora, A., Gurlebeck, D., Peart, J., Ganai, M., Baker, B., Bonas, U. and Lahaye, T. (2004) The tomato resistance protein *Bs4* is a predicted non-nuclear TIR-NB-LRR protein that mediates defense responses to severely truncated derivatives of *AvrBs4* and overexpressed *AvrBs3*. *Plant J.* **37**, 46–60.
- Schornack, S., Meyer, A., Romer, P., Jordan, T. and Lahaye, T. (2006) Gene-for-gene-mediated recognition of nuclear-targeted *AvrBs3*-like bacterial effector proteins. *J. Plant Physiol.* **163**, 256–272.
- Schornack, S., Minsavage, G.V., Stall, R.E., Jones, J.B. and Lahaye, T. (2008) Characterization of *AvrHah1*, a novel *AvrBs3*-like effector from *Xanthomonas gardneri* with virulence and avirulence activity. *New Phytol.* **179**, 546–556.
- Shiotani, H., Fujikawa, T., Ishihara, H., Tsuyumu, S. and Ozaki, K. (2007) A *pthA* homolog from *Xanthomonas axonopodis* pv. *citri* responsible for host-specific suppression of virulence. *J. Bacteriol.* **189**, 3271–3279.
- Silipo, A., Molinaro, A., Sturiale, L., Dow, J.M., Erbs, G., Lanzetta, R., Newman, M.A. and Parrilli, M. (2005) The elicitation of plant innate immunity by lipooligosaccharide of *Xanthomonas campestris*. *J. Biol. Chem.* **280**, 33660–33668.
- da Silva, A.C., Ferro, J.A., Reinach, F.C., Farah, C.S., Furlan, L.R., Quaggio, R.B., Monteiro-Vitorello, C.B., Van Sluys, M.A., Almeida, N.F., Alves, L.M., do Amaral, A.M., Bertolini, M.C., Camargo, L.E., Camarotte, G., Cannavan, F., Cardozo, J., Chambergo, F., Ciapina, L.P., Cicarelli, R.M., Coutinho, L.L., Cursino-Santos, J.R., El Dorry, H., Faria, J.B., Ferreira, A.J., Ferreira, R.C., Ferro, M.I., Formighieri, E.F., Franco, M.C., Greggio, C.C., Gruber, A., Katsuyama, A.M., Kishi, L.T., Leite, R.P., Lemos, E.G., Lemos, M.V., Locali, E.C., Machado, M.A., Madeira, A.M., Martinez-Rossi, N.M., Martins, E.C., Meidanis, J., Menck, C.F., Miyaki, C.Y., Moon, D.H., Moreira, L.M., Novo, M.T., Okura, V.K., Oliveira, M.C., Oliveira, V.R., Pereira, H.A., Rossi, A., Sena, J.A., Silva, C., de Souza, R.F., Spinola, L.A., Takita, M.A., Tamura, R.E., Teixeira, E.C., Tezza, R.I., Trindade dos, S.M., Truffi, D., Tsai, S.M., White, F.F., Setubal, J.C. and Kitajima, J.P. (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*, **417**, 459–463.
- Song, W.Y., Wang, G., Chen, L., Kim, H., Pi, L., Holsten, T., Gardner, J., Wang, B., Zhai, W., Zhu, L., Fauquet, C. and Ronald, P. (1995) A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science*, **270**, 1804–1806.
- Sory, M.P., Boland, A., Lambermont, I. and Cornelis, G.R. (1995) Identification of the YopE and YopH domains required for secretion and internalization into the cytosol of macrophages, using the *cyaA* gene fusion approach. *Proc. Natl. Acad. Sci. USA*, **92**, 11998–12002.
- Starr, M.P. (1983) *The Genus Xanthomonas*. New York: Springer-Verlag.
- Stavrinos, J., Ma, W. and Guttman, D.S. (2006) Terminal reassortment drives the quantum evolution of type III effectors in bacterial pathogens. *PLoS Pathog.* **2**, e104.
- Sugio, A., Yang, B., Zhu, T. and White, F.F. (2007) Two type III effector genes of *Xanthomonas oryzae* pv. *oryzae* control the induction of the host genes *OstFIIAγ1* and *OstTFX1* during bacterial blight of rice. *Proc. Natl. Acad. Sci. USA*, **104**, 10720–10725.
- Sun, W., Dunning, F.M., Pfund, C., Weingarten, R. and Bent, A.F. (2006) Within-species flagellin polymorphism in *Xanthomonas campestris* pv. *campestris* and its impact on elicitation of Arabidopsis FLAGELLIN SENSING2-dependent defenses. *Plant Cell*, **18**, 764–779.
- Sun, X., Cao, Y., Yang, Z., Xu, C., Li, X., Wang, S. and Zhang, Q. (2004) *Xa26*, a gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* in rice, encodes an LRR receptor kinase-like protein. *Plant J.* **37**, 517–527.
- Swarup, S., De Feyter, R., Brlansky, R.H. and Gabriel, D.W. (1991) A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit canker-like lesions on citrus. *Phytopathology*, **81**, 802–809.
- Swords, K.M.M., Dahlbeck, D., Kearney, B., Roy, M. and Staskawicz, B.J. (1996) Spontaneous and induced mutations in a single open reading frame alter both virulence and avirulence in *Xanthomonas campestris* pv. *vesicatoria* *avrBs2*. *J. Bacteriol.* **178**, 4661–4669.
- Szurek, B., Marois, E., Bonas, U. and Van Den, A.G. (2001) Eukaryotic features of the *Xanthomonas* type III effector *AvrBs3*: protein domains

- involved in transcriptional activation and the interaction with nuclear import receptors from pepper. *Plant J.* **26**, 523–534.
- Tai, T.H., Dahlbeck, D., Clark, E.T., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E. and Staskawicz, B.J. (1999) Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl. Acad. Sci. USA*, **96**, 14153–14158.
- Thieme, F. (2008) Genombasierte identifizierung neuer potentieller Virulenzfaktoren von *Xanthomonas campestris* pv. *vesicatoria*. Thesis, Mathematisch–Naturwissenschaftlich–Technischen Fakultät der Martin-Luther Universität, Halle-Wittenberg. [WWW document]. URL <http://sundoc.bibliothek.uni-halle.de/diss-online/06/06H103/prom.pdf> [accessed on Sep. 29, 2009].
- Thieme, F., Koebnik, R., Bekel, T., Berger, C., Boch, J., Buttner, D., Caldana, C., Gaigalat, L., Goesmann, A., Kay, S., Kirchner, O., Lanz, C., Linke, B., McHardy, A.C., Meyer, F., Mittenhuber, G., Nies, D.H., Niesbach-Klosgen, U., Patschkowski, T., Ruckert, C., Rupp, O., Schneiker, S., Schuster, S.C., Vorholter, F.J., Weber, E., Puhler, A., Bonas, U., Bartels, D. and Kaiser, O. (2005) Insights into genome plasticity and pathogenicity of the plant pathogenic bacterium *Xanthomonas campestris* pv. *vesicatoria* revealed by the complete genome sequence. *J. Bacteriol.* **187**, 7254–7266.
- Thieme, F., Szczesny, R., Urban, A., Kirchner, O., Hause, G. and Bonas, U. (2007) New type III effectors from *Xanthomonas campestris* pv. *vesicatoria* trigger plant reactions dependent on a conserved N-myristoylation motif. *Mol. Plant–Microbe Interact.* **20**, 1250–1261.
- Turton, K., Chaddock, J.A. and Acharya, K.R. (2002) Botulinum and tetanus neurotoxins: structure, function and therapeutic utility. *Trends Biochem. Sci.* **27**, 552–558.
- Ulrich, H.D. (2009) The SUMO system: an overview. *Methods Mol. Biol.* **497**, 3–16.
- Van den Ackerveken, G., Marois, E. and Bonas, U. (1996) Recognition of the bacterial avirulence protein AvrBs3 occurs inside the host plant cell. *Cell*, **87**, 1307–1316.
- Vorholter, F.J., Schneiker, S., Goesmann, A., Krause, L., Bekel, T., Kaiser, O., Linke, B., Patschkowski, T., Ruckert, C., Schmid, J., Sidhu, V.K., Sieber, V., Tauch, A., Watt, S.A., Weisshaar, B., Becker, A., Niehaus, K. and Puhler, A. (2008) The genome of *Xanthomonas campestris* pv. *campestris* B100 and its use for the reconstruction of metabolic pathways involved in xanthan biosynthesis. *J. Biotechnol.* **134**, 33–45.
- Wang, L., Tang, X. and He, C. (2007) The bifunctional effector AvrXccC of *Xanthomonas campestris* pv. *campestris* requires plasma membrane-anchoring for host recognition. *Mol. Plant Pathol.* **8**, 491–501.
- Wei, C.F., Kvitko, B.H., Shimizu, R., Crabill, E., Alfano, J.R., Lin, N.C., Martin, G.B., Huang, H.C. and Collmer, A. (2007) A *Pseudomonas syringae* pv. *tomato* DC3000 mutant lacking the type III effector HopQ1-1 is able to cause disease in the model plant *Nicotiana benthamiana*. *Plant J.* **51**, 32–46.
- Whalen, M.C., Richter, T., Zakharevich, K., Yoshikawa, M., Al-Azzeh, D., Adefioye, A., Spicer, G., Mendoza, L.L., Morales, C.Q., Klassen, V., Perez-Baron, G., Toebe, C.S., Tzovolous, A., Gerstman, E., Evans, E., Thompson, C., Lopez, M. and Ronald, P.C. (2008) Identification of a host 14-3-3 protein that interacts with *Xanthomonas* effector AvrRxv. *Phys. Mol. Plant Pathol.* **72**, 46–55.
- Whalen, M.C., Wang, J.F., Carland, F.M., Heiskell, M.E., Dahlbeck, D., Minsavage, G.V., Jones, J.B., Scott, J.W., Stall, R.E. and Staskawicz, B.J. (1993) Avirulence gene *avrRxv* from *Xanthomonas campestris* pv. *vesicatoria* specifies resistance on tomato line Hawaii 7998. *Mol. Plant–Microbe Interact.* **6**, 616–627.
- White, F.F. and Yang, B. (2009) Host and pathogen factors controlling the rice/*Xanthomonas oryzae* interaction. *Plant Physiol.* **150**, 1677–1686.
- Wichmann, G. and Bergelson, J. (2004) Effector genes of *Xanthomonas axonopodis* pv. *vesicatoria* promote transmission and enhance other fitness traits in the field. *Genetics*, **166**, 693–706.
- Xiang, Y., Cao, Y., Xu, C., Li, X. and Wang, S. (2006) *Xa3*, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26*. *Theor. Appl. Genet.* **113**, 1347–1355.
- Xu, R.Q., Blanvillain, S., Feng, J.X., Jiang, B.L., Li, X.Z., Wei, H.Y., Kroj, T., Lauber, E., Roby, D., Chen, B., He, Y.Q., Lu, G.T., Tang, D.J., Vasse, J., Arlat, M. and Tang, J.L. (2008) AvrAC(Xcc8004), a type III effector with a leucine-rich repeat domain from *Xanthomonas campestris* pathovar *campestris* confers avirulence in vascular tissues of *Arabidopsis thaliana* ecotype Col-0. *J. Bacteriol.* **190**, 343–355.
- Yang, B., Sugio, A. and White, F.F. (2005) Avoidance of host recognition by alterations in the repetitive and C-terminal regions of AvrXa7, a type III effector of *Xanthomonas oryzae* pv. *oryzae*. *Mol. Plant–Microbe Interact.* **18**, 142–149.
- Yang, B., Sugio, A. and White, F.F. (2006) *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *Proc. Natl. Acad. Sci. USA*, **103**, 10503–10508.
- Yang, B. and White, F.F. (2004) Diverse members of the AvrBs3/PthA family of type III effectors are major virulence determinants in bacterial blight disease of rice. *Mol. Plant–Microbe Interact.* **17**, 1192–1200.
- Yang, B., Zhu, W., Johnson, L.B. and White, F.F. (2000) The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent, nuclear-localized, double-stranded DNA binding protein. *Proc. Natl. Acad. Sci. USA*, **97**, 9807–9812.
- Yang, Y., De Feyter, R. and Gabriel, D.W. (1994) Host-specific symptoms and increased release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from leaves are determined by the 102-bp tandem repeats of *pthA* and *avrb6*, respectively. *Mol. Plant–Microbe Interact.* **7**, 345–355.
- Yang, Y. and Gabriel, D.W. (1995) *Xanthomonas* avirulence/pathogenicity gene family encodes functional plant nuclear targeting signals. *Mol. Plant–Microbe Interact.* **8**, 627–631.
- Yarbrough, M.L. and Orth, K. (2009) AMPylation is a new post-translational modification. *Nat. Chem. Biol.* **5**, 378–379.
- Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z., Kono, I., Yano, M., Iwata, N. and Sasaki, T. (1998) Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. *Proc. Natl. Acad. Sci. USA*, **95**, 1663–1668.
- Yuan, M., Chu, Z., Li, X., Xu, C. and Wang, S. (2009) Pathogen-induced expressional loss of function is the key factor of race-specific bacterial resistance conferred by a recessive R gene *xa13* in Rice. *Plant Cell Physiol.* **50**, 947–955.
- Zhao, B., Ardales, E.Y., Raymundo, A., Bai, J., Trick, H.N., Leach, J.E. and Hulbert, S.H. (2004) The *avrXo1* gene from the rice pathogen *Xanthomonas oryzae* pv. *oryzicola* confers a nonhost defense reaction on maize with resistance gene *Rxo1*. *Mol. Plant–Microbe Interact.* **17**, 771–779.
- Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J. and Hulbert, S. (2005) A maize resistance gene functions against bacterial streak disease in rice. *Proc. Natl. Acad. Sci. USA*, **102**, 15383–15388.

Zhu, W., Magbanua, M.M. and White, F.F. (2000) Identification of two novel hrp-associated genes in the hrp gene cluster of *Xanthomonas oryzae* pv. *oryzae*. *J. Bacteriol.* **182**, 1844–1853.

Zhu, W., Yang, B., Chittoor, J.M., Johnson, L.B. and White, F.F. (1998) AvrXA10 contains an acidic transcriptional activation domain in the

functionally conserved C terminus. *Mol. Plant–Microbe Interact.* **11**, 824–832.

Zhu, W., Yang, B., Wills, N., Johnson, L.B. and White, F.F. (1999) The C terminus of AvrXa10 can be replaced by the transcriptional activation domain of VP16 from the herpes simplex virus. *Plant Cell*, **11**, 1665–1674.