Xanthomonas diversity, virulence and plant–pathogen interactions

Sujan Timilsina[1,4], Neha Potnis[2,4], Eric A. Newberry[2], Prabha Liyanapathiranage[2], Fernanda Iruegas-Bocardo[1], Frank F. White[1], Erica M. Goss[1,3]✉ and Jeffrey B. Jones[1]✉

Abstract | *Xanthomonas* spp. encompass a wide range of plant pathogens that use numerous virulence factors for pathogenicity and fitness in plant hosts. In this Review, we examine recent insights into host–pathogen co-evolution, diversity in *Xanthomonas* populations and host specificity of *Xanthomonas* spp. that have substantially improved our fundamental understanding of pathogen biology. We emphasize the virulence factors in xanthomonads, such as type III secreted effectors including transcription activator-like effectors, type II secretion systems, diversity resulting in host specificity, evolution of emerging strains, activation of susceptibility genes and strategies of host evasion. We summarize the genomic diversity in several *Xanthomonas* spp. and implications for disease outbreaks, management strategies and breeding for disease resistance.

Vascular tissue
Tissue involved in transporting nutrients and fluids in plants. The primary components include xylem and phloem.

Mesophyll tissue
Leaf tissue between the epidermis layers that carries out photosynthesis.

Recombination
Genetic exchange between bacteria resulting in the incorporation of homologous and non-homologous sequences.

[1]*Plant Pathology Department, University of Florida, Gainesville, FL, USA.*

[2]*Entomology and Plant Pathology, Auburn University, Auburn, AL, USA.*

[3]*Emerging Pathogens Institute, University of Florida, Gainesville, FL, USA.*

[4]*These authors contributed equally: Sujan Timilsina, Neha Potnis.*

✉*e-mail: emgoss@ufl.edu; jbjones@ufl.edu*

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Xanthomonas is a Gram-negative bacterial genus in the class Gammaproteobacteria, and contains species causing diseases in more than 400 different plant hosts, such as rice, wheat, citrus, tomato, pepper, cabbage, cassava, banana and bean[1,2] (FIG. 1). Outbreaks of *Xanthomonas* diseases have been reported from multiple hosts worldwide[3,4]. Banana *Xanthomonas* wilt, which continues to spread in Central and East African countries, has caused major losses to banana production and threatens the livelihood of millions of farmers, who use it as both a food and a cash crop[5–7]. The genus has undergone changes in nomenclature over the past 25 years based on phenotypic and conventional molecular techniques and, more recently, whole-genome sequencing (WGS)[8–10]. The genus currently comprises more than 35 species[11] and is subdivided into subspecies or pathovars. Xanthomonads are characterized by a unique yellow pigment, xanthomonadin[12], although some strains do not produce this pigment, such as *X. axonopodis* pv. *manihotis*, *X. campestris* pv. *mangiferaindicae* and *X. campestris* pv. *viticola*[13–15]. Overall, studies have shown extensive genomic diversity among *Xanthomonas* spp. that can colonize unique ecological niches. For example, *X. albilineans* and *X. oryzae* pv. *oryzae* colonize the vascular tissue of sugarcane and rice, respectively, whereas many other *Xanthomonas* spp. preferentially colonize mesophyll tissue[2,16,17]. Additionally, comprehensive ecological studies have identified non-pathogenic *Xanthomonas* strains, which add to the previously estimated diversity in the genus[18]. Recombination and horizontal gene transfer contribute to the pathogen population structure and diversity across different *Xanthomonas* pathosystems[19–21]. Several factors, such as the type III secretion system (T3SS) and associated effectors, lipopolysaccharides, adhesins, transcription factors and TonB-dependent receptors, have been identified that influence host specificity and bacterial pathogenicity in several *Xanthomonas* spp.[22–24]. Among the widely prevalent and studied xanthomonads are pathovars of *X. oryzae*, causal agents of bacterial blight and leaf streak of rice; *Xanthomonas* spp. that cause bacterial spot disease in tomato and pepper; citrus canker caused primarily by *X. citri* pv. *citri*; and *X. arboricola*, pathogenic on stone fruits and nuts — therefore, these taxa will be the focus of this Review.

Xanthomonas spp. use the T3SS, encoded by the *hrp* cluster, to translocate proteins referred to as type III secreted effectors (T3SEs) into plant host cells[25,26]. *Xanthomonas* T3SEs are generally called Xops (*Xanthomonas* outer proteins), except for AvrBs1, AvrBs2 and AvrBs3, which are traditionally associated with their respective avirulence phenotype, recognized by corresponding R proteins from hosts, resulting in effector-triggered immunity (ETI)[26]. Currently, 53 Xop families are known, with an alphabetical nomenclature from XopA to XopBA (Overview of T3SEs in *Xanthomonas* Resource). These effectors have important roles in host colonization and pathogenicity. Improved genomic databases, population and genome-wide association studies, and machine-learning approaches have improved the identification of Xops and their interactions with the plant hosts, when the phenotype is indistinct[27,28]. The T3SS contributes significantly towards suppression of host defences and disease progression, and there has been considerable progress in our understanding of the contribution of other pathogenicity

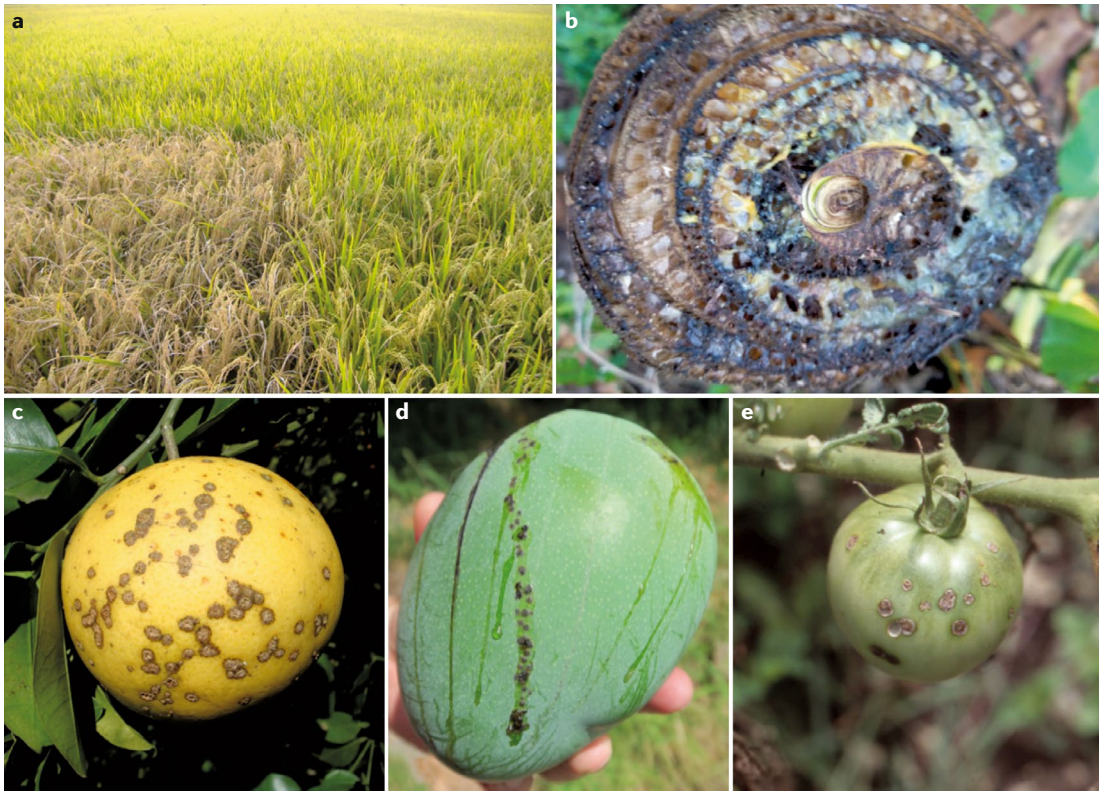


Fig. 1 | ***Xanthomonas* spp. in different plant hosts. a** | *X. oryzae* causes bacterial blight in rice. This species encompasses two pathovars, *oryzae* and *oryzicola*. **b** | *X. campestris* pv. *musacearum* causes banana *Xanthomonas* wilt, which can lead to extensive oozing. **c** | *X. citri* infects citrus and produces unique pustules in the leaf and fruit tissues. **d** | *X. axonopodis* pv. *mangiferaindicae* causes mango black spot disease. **e** | Four *Xanthomonas* spp. are associated with bacterial spot disease in tomato and pepper: *X. cynarae* pv. *gardneri*, *X. euvesicatoria*, *X. perforans* and *X. vesicatoria*. Part **b** courtesy of M. M. Shimwela, Tanzania Agricultural Research Institute, Maruku, Tanzania; part **c** courtesy of A. M. Gochez, National Agricultural Technology Institute, Argentina; all other images provided by authors (F.F.W and J.B.J).

Type III secretion system (T3SS). A secretion system composed of ~20 proteins that forms a syringe-like structure to deliver bacterial proteins to eukaryotic cells. Also referred to as the injectisome.

Effector-triggered immunity (ETI). Innate immune response triggered by recognition of the type III translocated effector proteins by host resistance gene products.

Type II secretion system (T2SS). A secretion system formed by secretin proteins, which form characteristic β-barrels for passage of secreted proteins.

Type VI secretion system (T6SS). A secretion system that delivers bacterial proteins across a cellular envelope to adjacent target cells. Primarily known for interbacterial antagonism.

factors, such as cell wall-degrading enzymes secreted by the type II secretion system (T2SS), type IV secreted effectors, the type VI secretion system (T6SS) and associated effectors, adhesins, lipopolysaccharides, small RNAs and regulators, such as Rpf, HrpG, HrpX, HpaR, Clp, Zur, FhrR and RsmA[29–35]. Not all of these other secretion systems and factors are directly involved in virulence of the pathogen but they can affect pathogen fitness[34].

Understanding plant–microorganism interactions within an ecological context has been key in developing new knowledge to enhance overall plant health. Recent studies of *Xanthomonas* spp. have integrated the host, pathogen and microbial community influencing disease development, making it a model system to study plant pathogenic bacteria. In this Review, we will cover recent insights into *Xanthomonas* spp. virulence factors, diversity and their evolution. We will highlight the genomic diversity in *Xanthomonas* spp., examine current understanding in pathogenomics and discuss mechanisms of host evasion.

Xanthomonas genomics and diversity

Starting with the sequencing of two *Xanthomonas* spp. in the early 2000s (REF.[36]), there are now more than 1,400 *Xanthomonas* genomes representing all named *Xanthomonas* spp. publicly available in the National Center for Biotechnology Information (NCBI) database. A typical *Xanthomonas* genome is ~5 Mb with a GC content well over 60% and encodes >4,000 genes[1,37,38]. The exception is *X. albilineans*, which has a reduced genome of ~3.7 Mb (REFS[39,40]). This species has undergone genome erosion with an estimated loss of more than 500 genes, but the drivers of this gene loss are unclear[39,41].

Xanthomonas diversity can be categorized at multiple levels, including genetic diversity within populations and species, and functional or ecological diversity, which describes their roles in plant microbiomes. Our understanding of *Xanthomonas* diversity is mainly based on population and species-level data, described by analyses of single genes, several housekeeping genes and whole genomes. Recent studies targeting microbial communities have revealed ecologically diverse lineages within *Xanthomonas* spp. and novel pathogenic and non-pathogenic species.

Population and species diversity. Advances in omics tools have revealed more of the *Xanthomonas* diversity and identified mechanisms of speciation and evolution[42,43]. Strains of *X. arboricola* pv. *juglandis* are increasingly reported from various parts of Europe and population studies have found unprecedented genetic diversity, with non-pathogenic strains cohabiting with

Hypersensitive response
A response mechanism found in plant hosts, characterized typically by a rapid cell death to prevent the spread of the pathogen.

Accessions
Groups of related plant material from the same species collected from a specific location. The accessions are collections to capture the diversity in a given plant species.

pathogenic strains in several plants[18,44,45]. Genomic comparisons found that several non-pathogenic strains of *X. arboricola* and *X. cannabis* carried only four T3SS-associated regulatory genes (*hrpG*, *hrpX*, *hpaS* and *hpaR2*) and orthologues of six T3SEs, compared with 24 orthologues found in pathogenic strains[45,46].

High genome plasticity has been shown in several *Xanthomonas* spp., suggesting mechanisms for bacterial adaptability and response to selection. Comparison of bacterial spot-causing xanthomonads in tomato and pepper showed genome-wide recombination of *X. perforans* with *X. euvesicatoria*[19,47,48]. A host-driven population shift was observed in *X. oryzae* pv. *oryzae*. Six different groups with distinct genotypic characteristics evolved after introduction of plants with the *Xa4* resistance locus in the Philippines[49]. A total of 386 full-length insertion sequences was found in a single genome of *X. oryzae*, indicating high genome plasticity of individual strains[50]. Insertion sequences are reported to have an important role in genome instability and loss of gene function, as exemplified by the insertion sequence-mediated inactivation of the *gumM* gene, which is involved in xanthan production, and thus abrogated the production of this extracellular polysaccharide in *X. oryzae*[51]. Plasmids are another source of genomic variability and *Xanthomonas* diversity, and a chimeric (hybrid) plasmid was reported in an *X. citri* pv. *citri* strain carrying four copies of the same type of effectors[52] in a single plasmid[53]. Similarly, mobile elements including integrative and conjugative elements, which typically function as conjugative transposons, have been reported to carry copper resistance genes in the pathogenic *X. arboricola* strain CFBP 7179 (REF.[18]).

Intraspecific diversity and host specialization are apparent in *X. citri* pv. *citri*, which exhibits three pathotypes: A, A* and A^w. These three pathotypes have a varying host range: pathotype A has a wide host range, whereas A* only infects *Citrus aurantifolia*, *C. latifolia* and *C. macrophylla*, and A^w affects *C. aurantifolia and C. macrophylla*[54]. A^w also induces a hypersensitive response in grapefruit[54,55]. The effector AvrGf1 found in A^w was identified as a host-limiting factor that determined the hypersensitive response in grapefruit[56]. Although deletion of this gene in A^w resulted in no hypersensitive response, the mutated strain was unable to grow to levels similar to an A-type strain, thus suggesting the presence of additional host-limiting factor(s) in distinct lineages. A recent WGS study of 95 *X. citri* pv. *citri* strains predicted that the diversification of these strains occurred approximately 1,700–5,700 years ago[57]. This diversification coincides with the spread of citrus cultivars in Asia, much later than the origin of citrus, suggesting that the pathotypes evolved as a result of cross-infection by dispersal rather than by host-driven speciation[57]. Unlike *X. citri* pv. *citri* diversification, a host-driven population shift was observed in *X. oryzae* pv. *oryzae* in response to the introduction of resistant plants[49].

Diversity in the phytobiome. Comprehensive studies of *Xanthomonas* diversity and epidemiology should take into account the total microbial population. An analysis of the leaf microbiomes of 3,024 rice accessions adapted to a wide variety of agro-ecosystems in China and Philippines, two major rice production areas, found that the leaf microbiome converged to a few central taxa that strongly regulated the microbial networks[58]. *Xanthomonas* was among the most abundant genera within these microbiomes. Another study investigated the seed microbiomes of five genotypes of rice and found that *Xanthomonas* was one of the abundant genera shared by all of the genotypes[59], indicating that they form part of the major core of endophytic bacteria. However, the role of *Xanthomonas* spp. as dominant endophytes in healthy, asymptomatic rice seeds is not well understood. This type of study begins to unravel the ecological roles of *Xanthomonas* spp. within the host microbiome (BOX 1).

Virulence mechanisms

Evolution of Xanthomonas-associated secretion systems. WGS of diverse *Xanthomonas* spp. enabled studying the evolution of the *Xanthomonas* core genome. Phylogenomic analysis of the core genome indicates two major groups within *Xanthomonas*, and at least five clades within group 2 (FIG. 2). WGS has also provided insights into the evolution of secretion systems, their associated virulence factors and their ancestral acquisition patterns (FIG. 2). The T3SS cluster, also known as the Hrp (hypersensitive response and pathogenicity)

Box 1 | Host specificity and ecology of *Xanthomonas* spp.

Xanthomonas spp. encompass a large group of plant-associated bacteria that usually cause disease. Recently, there has been an increased focus on ecological and genomic studies of xanthomonads[2,21]. Although members in this genus are found on a wide variety of hosts, including monocots and dicots, species are associated with a limited number of hosts and produce either localized or systemic infections. Host specificity has been suggested as the main species determinant in xanthomonads[175]. As avirulence genes were identified in different *Xanthomonas* spp., they were regarded as determinants of host range and defined pathovar nomenclature in xanthomonads. As we have gained an appreciation of diversity of xanthomonads through sampling efforts, whole-genome sequencing and functional analyses, effectors were suggested to be involved in defining the host range by the repertoire-for-repertoire hypothesis[176]. Comparative genomic studies of *Xanthomonas* strains have identified several candidate virulence factors with a major role in host specificity and tissue localization. However, genomic analyses have also revealed a complex interplay of multiple factors underlying host and tissue specificity. A population-based analysis of 67 *Xanthomonas* genomes identified *avrBsT* and *xopQ* as host-limiting effectors in *X. perforans*[43]. Nonetheless, deletion of these genes from different phylogenetic groups of *X. perforans* did not always result in a broader host range. Growing evidence has suggested that host specificity in xanthomonads is not limited to T3SEs or repertoires of T3SEs; instead multiple genetic determinants underlie this process and the effects of individual genes range from small to major[2,20,177].

Xanthomonas strains show high tissue specificity, but the genetic drivers of this specificity are as yet unknown. A study compared vascular and non-vascular strains of *Xanthomonas* spp. from both monocots and dicots to unravel the determinants of tissue specificity[178]. *X. albilineans*, a vascular pathogen of sugarcane, has a reduced genome compared with other xanthomonads and lacks the extracellular polysaccharide gum gene cluster and the type III secretion system[41]. Comparative genomics have been used to identify the putative gene(s) responsible for tissue specificity. Preliminary studies have indicated the role of cellobiosidase, CelA and/or CbhA, in vascular colonization[46,179]. Additionally, with more microbial community studies, *Xanthomonas* spp. have been found to colonize previously unpredicted ecological niches. The approaches for microbial studies are shifting from selective genotyping of strains to population and community studies in multiple host–pathogen systems[2,180]. Comprehensive studies that consider potentially diverse functional and ecological roles are required to generate knowledge on the complex systems driving *Xanthomonas* spp. pathogenicity and host and tissue specificity.

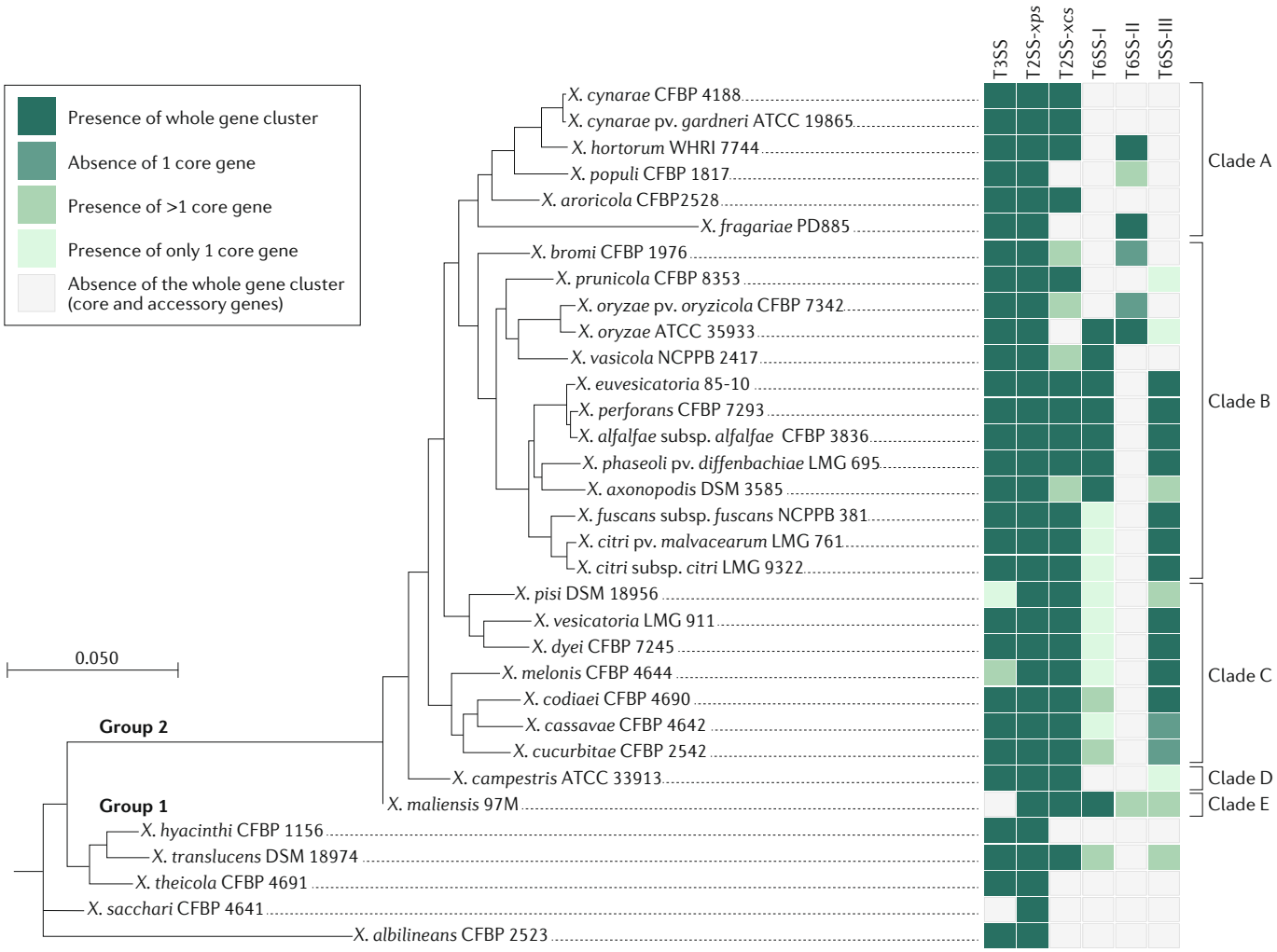


Fig. 2 | **Diversity of *Xanthomonas* spp. and lineages and their virulence genes.** We determined the phylogenetic distribution of *Xanthomonas* spp. based on the core alignment of 198,114 nucleotide sequences using the Roary pipeline[171]. Whole-genome sequences of type strains or completely sequenced genomes representing the *Xanthomonas* spp. available in the National Center for Biotechnology Information (NCBI) database were used for phylogenetic reconstruction. The presence of virulence-associated secretion systems is shown. The *xps* type II secretion system (T2SS) is conserved in all *Xanthomonas* spp. The type III secretion system (T3SS) is found in most strains, except *X. maliensis* and *X. sacchari*. *X. hyacinthi*, *X. theicola* and *X. albilineans* have an atypical T3SS. Of the group 1 strains, only *X. translucens* carries the full T3SS. *X. pisi* and *X. dyei* have more than one core gene of the T3SS. T6SS, type VI secretion system.

cluster, which belongs to the Hrp2 family in the genus *Xanthomonas*, has been extensively studied. For all group 2 species except *X. campestris*, acquisition of the Hrp2 cluster occurred in their common ancestor[60]. *X. campestris* pv. *campestris* independently acquired Hrp2, as suggested by the chromosomal location of its T3SS cluster, which differs from the other group 2 species[46]. A different genetic organization, different genomic content and high divergence at the sequence level of the Hrp2 cluster in group 1 species compared with group 2 species indicated independent acquisition in group 1 (REF.[60]). Some species and strains that are scattered throughout the phylogenetic tree seem to have lost the Hrp2 cluster. Gene flow in the Hrp2 cluster was observed between *X. arboricola* strains belonging to clade A and *X. dyei* and *X. hortorum*[60].

Although the T3SS is considered the primary secretion system responsible for virulence, the contribution of other secretion systems, including the T2SS and T6SS, towards pathogenesis or overall pathogen fitness has been shown in *X. euvesicatoria*, *X. citri* pv. *citri* and *X. oryzae* pv. *oryzae*[30,61–63]. These two systems have been implicated in the secretion of several cell wall-degrading enzymes. The T2SS Xps cluster is conserved in the *Xanthomonas* genus. Furthermore, the T2SS Xcs cluster is present in all clade C, D and E strains and in most clade A and B strains, but is absent from *X. populi*, *X. fragarie* and *X. oryzae* strains. *X. arboricola*, *X. vasicola*, *X. oryzae* pv. *oryzicola* and *X. bromi* had partial Xcs clusters. The Xcs cluster is absent from group 1 strains, with the exception of *X. translucens* (FIG. 2). The T6SS is important for interactions with both prokaryotic and eukaryotic neighbours, including manipulation of virulence in animal pathogens[64,65]. In plant pathogens, there is little evidence for its direct interaction with the plant hosts but it

Pathogen or damage-associated molecular pattern (P/DAMP)-triggered immunity (PTI/DTI). PTI refers to the immune response in hosts triggered by recognizing patterns associated with pathogen, for example, flagellin or lipopolysaccharide. DTI refers to the host immune response triggered as a result of recognition of cell wall-degradation products that are generated by the action of pathogen-secreted cell wall-degrading enzymes during pathogen invasion. PTI and DTI pathways have a significant overlap in their signalling components.

Receptor-like cytoplasmic kinases
Kinase-mediated signalling proteins that regulate plant cellular activities in response to biotic or abiotic stresses and endogenous extracellular signalling molecules.

Receptor-like kinase superfamily
Transmembrane proteins with versatile amino-terminal extracellular domains and carboxy-terminal intracellular kinases. They control a wide range of physiological responses in plants and belong to one of the largest gene families in the *Arabidopsis thaliana* genome, with more than 600 members.

MAPK
Protein kinases involved in regulating cellular responses to an extensive array of stimuli, including mitogens, heat shock and stress. Specific to serine and threonine amino acids.

Protoplast
The entire cell excluding the cell wall.

SWEET genes
Sugar will eventually be exported transporter (*SWEET*) genes encode membrane proteins with diverse function, typically facilitating sucrose and glucose efflux.

Recessive resistance
Resistance conferred by recessive allele of a gene in a plant host. The term is also used to refer to resistance conferred by mutation in disease-susceptibility genes.

influences interactions with other members of the plant microbiota[35,63]. The presence of the T6SS across different clades warrants attention to its role in xanthomonads. Based on the gene content and phylogeny, three different T6SSs are described in *Xanthomonas*. T6SS-I is present in some of the clade B strains, including *X. oryzae*, *X. vasicola*, species belonging to the *X. euvesicatoria* complex, *X. axonopodis* and *X. phaseoli*. *X. maliensis* is an exception as it has the complete T6SS-I cluster as well as partial clusters of T6SS-II and T6SS-III[63,66]. T6SS-II is present only in three species surveyed here: *X. hortorum*, *X. oryzae* and *X. fragarie*. T6SS-III is present in clade C, *X. euvesicatoria* and sister species, *X. citri* pv. *citri* and related species, and *X. phaseoli* from clade B (FIG. 2).

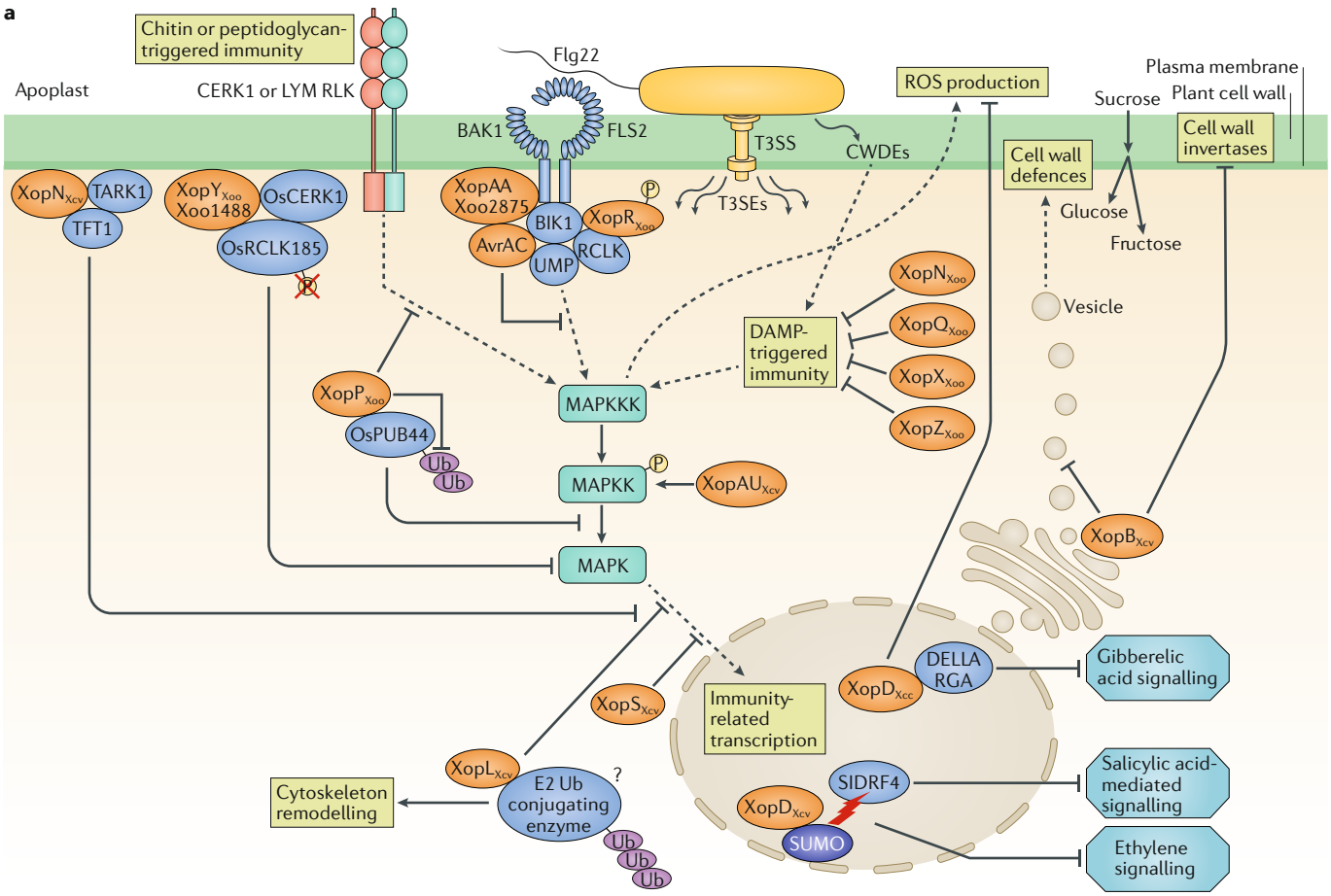
T3SS-dependent Xanthomonas outer proteins. T3SEs modulate host physiology to obtain nutrients, facilitate infection and/or evade host immune responses[67]. Putative identification of T3SEs has relied on homology-based searches largely driven by phenotypic observations, followed by functional reporter assays to confirm translocation of the candidate effectors into the plant cell[68,69]. These reporter assays take advantage of our understanding of molecular signals, including secretion and translocation signals found in T3SEs (REF.[68]). More recently, machine-learning approaches have been developed that rely on multiple criteria for the identification of novel effectors, such as secretion signals at the amino-terminus of T3SEs, amino acid composition, conserved motifs, structural disorder, regulation by HrpX and HrpG, GC content, codon use and homology to known and validated T3SEs (REFS[27,70]). Such a machine-learning approach identified seven novel T3SEs in *X. euvesicatoria* 85-10 as a representative genome and the method could be used to predict effectors from other Gram-negative bacteria that have a T3SS (REF.[27]).

T3SEs are integral to *Xanthomonas* pathogenicity, and are determinants of host specificity and pathogen fitness (FIG. 3). *Xanthomonas* effectors have evolved to target different components of the pathogen or damage-associated molecular pattern (P/DAMP)-triggered immunity (PTI/DTI) pathway[1,71]. The T3SEs $XopAC_{Xcc}$, $XopY_{Xoo}$, $XopAA_{Xoo}$ and $XopN_{Xe}$ target receptor-like cytoplasmic kinases, members of the receptor-like kinase superfamily. $XopQ_{Xoo}$, $XopX_{Xoo}$, $XopZ_{Xoo}$ and $XopN_{Xoo}$ inhibit DTI[72]. $XopAU_{Xe}$, a catalytically active protein kinase, promotes disease development by manipulating MAPK signalling through phosphorylation and activation of the immunity-associated MKK2 (REF.[73]). Examples of effectors that inhibit PTI include $XopP_{Xoo}$, $XopL_{Xe}$ and $XopS_{Xe}$[74,75]. Effectors such as AvrXv4, $XopJ_{Xe}$ and $XopD_{Xe}$ interfere with the host ubiquitin proteasomal system[76]. $XopB_{Xe}$ interferes with vesicle trafficking, interferes with cell wall-bound invertases and prevents sugar-mediated defence signals[77]. $XopD_{Xe}$, $XopD_{Xcc8004}$, $XopJ_{Xe}$ and XopAH (also known as AvrXccC) interfere with hormone signalling pathways involved in plant defences or disease susceptibility[78]. Effectors eliciting ETI have conventionally been identified as avirulence genes, and examples with known direct or indirect targets include XopJ4 (AvrXv4), XopH (AvrBs1.1), XopAG (AvrGf2), AvrXccC and AvrRxv[26,79]. T3SEs also function as ETI suppressors. Examples include AvrBsT, which is involved in suppression of AvrBs1-mediated ETI[80], and $XopQ_{Xe}$ (REF.[81]).

Several functional methods exist to characterize effectors in terms of their direct or indirect molecular targets in the host and their mode of action[69,82,83], including mutagenesis of the effector(s) and host interactor, *Agrobacterium tumefaciens*-mediated transient expression, yeast two-hybrid assays and pull-down assays. Two additional approaches, the protoplast transient expression assay[84] and the recently developed pathogen-free protoplast-based assay in *Arabidopsis thaliana*[85], were used to identify effectors that target specific host signalling pathways. For example, effectors from *X. euvesicatoria* 85-10 that interfere with PTI signalling mediated by Flg22, a highly conserved PAMP present in flagellin, were identified by expressing them in the attenuated *Pseudomonas syringae* pv. *tomato* DC3000ΔCEL strain[85]. Another method used to study *Xanthomonas* spp. effectors included using yeast as a heterologous system for expression of effectors and identifying effectors that affect cell growth and viability[86]. Recent technological advances in imaging tools enabled quantitative image-based phenotyping to study spatio-temporal dimensions of disease development for the vascular pathogen of cassava, *X. axonopodis* pv. *manihotis*, and to understand the contribution of individual effectors by time-resolved imaging[87].

TAL effectors. *Xanthomonas* spp. have evolved a distinct family of T3SEs known as transcription activation-like effectors (TALEs)[52], which increase plasticity in adaption of the bacteria to host plants. They have a rearrangeable repetitive domain that controls the ability to bind promoters of host susceptibility genes in a sequence-specific manner[88–90]. There is an uneven distribution of genes encoding TALEs among *Xanthomonas* spp. In some *Xanthomonas* spp., such as *X. gardneri*, *X. campestris*[36], *X. euvesicatoria*[91] and *X. perforans*[43], TALEs are not found in all strains, whereas TALEs are prevalent in *X. oryzae*, with *X. oryzae* pv. *oryzicola* strain BLS256 carrying a record 27 genes[37].

Various TALE-associated susceptibility genes, defined here as host genes associated with some aspect of disease or pathogen population, have been identified. A prominent example of TALEs and their cognate susceptibility are TALEs of *X. oryzae* pv. *oryzae* and *SWEET* genes of rice, which are responsible for a pronounced phenotype in bacterial blight of rice (BOX 2). Eight major TALEs are known in *X. oryzae* pv. *oryzae* that target one of three *SWEET* alleles of the clade III *SWEET* members; host targets that are convergently activated by multiple TALEs are referred to as susceptibility hubs[92–94]. In the absence of *SWEET* gene expression, bacteria fail to effectively colonize rice leaves. The TALE PthXo1 occurs in a subset of strains in the Asian lineage of *X. oryzae* pv. *oryzae*[89] and targets *SWEET11*, a sugar transporter gene that is essential for the early stage of rice grain filling[95]. Some rice cultivars have a recessive resistance allele (*xa13*), which interferes with PthXo1 function at the *SWEET11* promoter[89,96] (FIG. 4). Loss of function at a particular *SWEET* allele and consequential loss of bacterial virulence can be overcome by the presence


a
Chitin or peptidoglycan-triggered immunity
Apoplast
CERK1 or LYM RLK
Flg22
BAK1
FLS2
T3SS
T3SEs
CWDEs
ROS production
Plasma membrane
Plant cell wall
Sucrose
Cell wall invertases
Cell wall defences
Glucose
Fructose
Vesicle
XopN$_{Xcv}$
TARK1
TFT1
XopY$_{Xoo}$ Xoo1488
OsCERK1
OsRCLK185
XopAA Xoo2875
BIK1
AvrAC
UMP
RCLK
XopR$_{Xoo}$
XopN$_{Xoo}$
XopQ$_{Xoo}$
XopX$_{Xoo}$
XopZ$_{Xoo}$
DAMP-triggered immunity
XopP$_{Xoo}$
OsPUB44
Ub
MAPKKK
MAPKK
MAPK
XopAU$_{Xcv}$
XopB$_{Xcv}$
XopS$_{Xcv}$
Immunity-related transcription
XopD$_{Xcc}$
DELLA RGA
Gibberelic acid signalling
XopL$_{Xcv}$
E2 Ub conjugating enzyme
Cytoskeleton remodelling
SIDRF4
XopD$_{Xcv}$
SUMO
Salicylic acid-mediated signalling
Ethylene signalling
b
Apoplast
Flg22
BAK1
FLS2
T3SS
T3SEs
Plant cell wall
Plasma membrane
XopJ$_{Xcv}$
RPT6
Proteasome
AvrXccC
XopAH
?
ETI in Col1 *Arabidopsis* WT
In susceptible host
BIK1
AvrAC
PBL2
UMP
RKS1
ZAR1
AvrBsT
CaADC1
PA/GABA
XopB
ETI in pepper
Salicylic acid signalling
ABA homeostasis and signalling
MAPKKK
MEK2
WIPK
SIPK
CaPIK1
AvrBsT
CaSGT1
AvrBsT
CaALDH1
Ethylene signalling
Jasmonate signalling
AvrBsT
SRNK1
ETI in pepper
XopH
InsP$_6$
InsP$_5$
AvrBs1
ETI in *Bs1*-resistant pepper
Immunity-related transcription
ETI in *Bs7*-resistant pepper
AvrBs2
?
ETI in *Bs2*-resistant pepper
ETI in grapefruit
XopQ$_{Xcv}$
TFT4
XopAG
Chloroplast
AvrRxv
TFT9
ETI in H7998 tomato

◀ Fig. 3 | ***Xanthomonas* spp. effectors and their modes of action to trigger or suppress host defence responses. a** | Effectors involved in suppression of pathogen or damage-associated molecular pattern (P/DAMP)-triggered immunity can interact with receptor-like kinases (RLKs) or interfere with downstream signalling pathways. Some effectors can interfere with hormone signalling pathways, cytoskeleton remodelling or sugar-mediated defences during early pathogenesis. **b** | Avirulence genes and their products interact with specific host components in the cytoplasm, nucleus or chloroplast and trigger effector-triggered immunity (ETI). *Xanthomonas* spp. have evolved some effectors that can suppress the ETI response by direct or indirect interaction with the ETI components, or by modulating hormone signalling pathways. These effectors contribute to effector-triggered susceptibility. Please see Supplementary Box 1 for details on functions of individual effectors. CWDE, cell wall-degrading enzyme; P, phosphorylation; ROS, reactive oxygen species; T3SE, type III secreted effector; T3SS, type III secretion system; Ub, ubiquitination; WT, wild type; Xop, *Xanthomonas* outer protein.

of major TALEs that target other *SWEET* genes[97,98]. African lineage strains of *X. oryzae* pv. *oryzae* have evolved in apparent isolation from Asian lineage strains and have a distinct set of major TALEs that target *SWEET14*, which encodes a low-affinity sugar transporter[93,94,99,100].

Bacterial leaf streak is a wheat disease caused by *X. translucens* pv. *undulosa*, and one of eight genes encoding TALEs in the bacterial genome is associated with lesion length and the specific induction of the gene encoding 9-*cis*-epoxycarotenoid dioxygenase (NCED), causing a rise in the levels of the phytohormone abscisic acid[101]. A second *TALE* gene of *X. translucens* pv. *undulosa* with an unknown host target gene has been associated with virulence[102]. *Lateral organ boundaries 1* (*CsLOB1*), a member of the plant-specific lateral organ boundaries domain (LBD) family of transcription factor genes, is targeted for expression by several TALEs of *X. citri* pv. *citri* and *X. fuscans* pv. *aurantifolii*, the causal agents of citrus canker[103,104]. Loss of the ability to induce *CsLOB1* either by loss of the relevant TALE or modification of the effector binding site in the *CsLOB1* promoter by genome editing leads to loss of the typical canker symptoms[103,105,106].

TALE-mediated ETI involving nucleotide binding, leucine-rich repeat (NLR) resistance genes has been identified in tomato and rice[107–109]. Remarkably, the rice NLR gene, *Xa1*, was identified some time ago but research failed to identify the corresponding elicitor[110]. XA1, in fact, recognizes several TALEs, and most strains of *X. oryzae* have several TALEs. However, TALE-triggered resistance by XA1 is masked by sets of truncated TALEs, the iTALEs, which interfere with *Xa1* function and occur in most strains of *X. oryzae*[109]. For example, the iTALE Tal2h suppresses recognition mediated by rice *Xa1*. Similar findings have been reported for the NLR gene *Xo1* of the American heirloom rice variety Carolina Gold Select[40,108]. TALE-mediated ETI can also be triggered by host genes that combine an effector binding site with a gene encoding a toxic gene product or a so-called executor gene[111] (FIG. 4).

Other factors associated with fitness and virulence. A previous review has discussed in detail virulence factors such as extracellular polysaccharides, lipopolysaccharides, adhesins, substrates of virulence-associated secretion systems, including T1SS and T2SS, and the regulatory network, including RpfC, RpfG, RpfF, RavS, RavR, ColS, ColR, PhoP, PhoQ, Clp, Zur, FhrR, HrpX, HrpG and HpaR, and post-transcriptional control by RsmA[34]. The importance of small non-coding RNAs has been highlighted recently in *X. euvesicatoria*, *X. campestris* pv. *campestris* and *X. oryzae* pv. *oryzae*[112]. Several functional studies with vascular as well as non-vascular xanthomonads have indicated the importance of a repertoire of cell wall-degrading enzymes for virulence, although effects vary with the pathosystem and some show minimal contribution to overall virulence. Interestingly, cell wall-degrading enzymes, specifically xylanases, are secreted by outer membrane vesicles (OMVs) in *X. euvesicatoria*[113]. OMVs have also been called a type zero secretion system[114]. T3SEs could also be transported through OMVs or function in coordination with them. About half of the *X. campestris* pv. *campestris* OMV proteome consisted of virulence-associated proteins[115]. How these OMVs and associated virulence factors contribute to pathogenesis remains to be explored. Post-translational regulation of HrpG was recently demonstrated, in which stabilization of HrpG relied on host-induced phosphorylation of the ATP-dependent Lon protease[116]. A novel regulator, designated TfmR (T3SS and fatty acid mechanism regulator), was responsible for the upstream regulation of the T3SS in *X. citri* pv. *citri*[117]. The study also showed that fatty acids can have an important role in metabolic regulation of HrpG and HrpX. A two-component system (TCS), which consists of membrane-bound histidine kinase and a cytosolic response regulator, has an important role in niche adaptation of *Xanthomonas* spp. In *X. citri* pv. *citri*, cyclic di-GMP binds to RavS, which in turn induces phosphotransfer to RavR. The interaction between RavS and RavR, through a series of events, results in modulation of phosphorylation levels of RavS, which in turn is involved in switching between swimming and virulence, confirming the importance of this TCS in regulating lifestyles[118]. In another *X. citri* pv. *citri* strain, proteolysis of the histidine kinase VgrS prevents its autophosphorylation, which in turn promotes osmotolerance[119]. The histidine kinase PcrK can sense plant-derived stimuli, specifically the hormone cytokinin, which enables *X. citri* pv. *citri* to adapt to oxidative stress by regulating downstream genes including TonB-dependent receptor and other virulence-related genes[120]. Another TCS, involving StoS and SreKRS, regulates carbohydrate metabolism, chemotaxis, synthesis of extracellular polysaccharide and Hrp expression[121]. This TCS was proposed to contribute to fitness given its advantage in survival of *X. oryzae* pv. *oryzae* outside the host and overall adaptation[121]. XooNet is an in silico platform that has integrated genomic information to improve predictions of regulatory networks involving TCSs associated with virulence in *X. oryzae* pv. *oryzae*[122]. Other secretion systems that have not been discussed here in detail include the type IV secretion system and the T6SS. The type IV secretion system, and its effectors, and the T6SS have been characterized for their role in mediating xanthomonad interactions with the surrounding microbial community[29,62,63,123]. These interspecies and community level interactions need to be further explored to evaluate their contribution towards overall pathogen fitness.

Abscisic acid
A plant hormone with numerous functions in the plant developmental process, including dormancy and stress response.

Nucleotide binding, leucine-rich repeat (NLR) resistance genes
Resistance genes named after their characteristic nucleotide binding and leucine-rich repeat domains.

Two-component system
(TCS). Mediators of signal transduction in bacteria to detect the surrounding changes and relay the signal for modulating gene expression.

Box 2 | **Host targets of TALEs**

Candidate targets for Xanthomonas TALEs

Many type III secreted effectors (T3SEs) have been shown or are predicted to interfere with host immunity. How transcription activation-like effectors (TALEs) enhance host susceptibility, either by suppression of host immunity or other mechanisms, is largely unknown. Strains containing genes encoding TALEs also invariably harbour an array of other genes encoding T3SEs. Plant pathogenic species with genes encoding TALEs can suppress host immunity in the absence of TALEs. Loss of other effector genes has been correlated with reduced virulence in highly pathogenic strains of *X. oryzae*, which contain multiple genes encoding TALEs[72,83,148]. One difficulty is associating the loss of TALE function with changes in virulence. TALEs may promote expression of several genes due to a wobble in binding specificity. Without some indication of phenotype, assigning targeted genes to a function in susceptibility is largely conjectural, although rational. Most genes encoding TALEs, despite a few well-characterized members, have not been associated with phenotypes other than host gene transcription or effector-triggered immunity. As such, transcriptomic approaches have been applied to determine the candidate targets that are upregulated in the presence of the TALEs[103]. Following the identification of potential candidates, the promoter regions with potential binding sites are predicted based on the repeat variable di-residues and the TALE binding code. The elevated expression of the target host genes is quantified using real-time PCR of the mRNA genes with a housekeeping gene from the host. Microarray analyses identified *lateral organ boundaries 1* (*CsLOB1*) as a susceptibility gene for citrus canker. *CsLOB1* is a transcription factor in the lateral organ boundaries domain (LBD) family. Members of this family are often involved in tissue differentiation and maintenance of organ boundaries through both elevation and repression of downstream gene expression.

TALEs are more than susceptibility enhancing factors

Quite the opposite effect has been proposed for Brg11, which is one of the few members of the TALE family found outside *Xanthomonas* spp.[181]. Brg11 of *Ralstonia solanacearum* targets the gene for arginine decarboxylase (*ADC*), increasing putrescine levels and, consequentially, higher-order polyamines[182]. The rise in polyamines triggers a subset of defence response genes in the tomato host, which is proposed to reduce co-infection with other competing microorganisms. Thus, Brg11, instead of inducing a susceptibility gene, induces a niche-enhancing gene. A modified Brg11 introduced into *X. euvesicatoria* inhibited the growth of *Pseudomonas syringae* in co-infections of tomato[182]. Here, the focus has been on TALEs and genes with links to disease phenotypes, and Brg11 invites expansion of analyses of TALE function. A related family member is induced during root infection of *Arabidopsis thaliana* by the fungal pathogen *Fusarium oxysporum*, and a mutant plant for AtLBD20 has enhanced resistance to infection. A subset of genes in the jasmonic acid disease defence pathways were repressed in concert with AtLBD20 expression. The possibility exists, therefore, that *CsLOB1* is also involved in defence gene expression. In addition, tools are available to inform recent studies of TALE function, and associations of TALE-targeted genes with plant physiological insights will provide novel insights into TALE contributions[183–185].

Virulence evolution

Horizontal gene transfer and mutation of avirulence genes to evade host resistance are among the major factors that influence the evolution of virulence in *Xanthomonas* spp.[22,124,125]. As shown with several methods, approximately 5–25% of the genome of *Xanthomonas* spp. is acquired via recombination[126,127].

Comparison between pathogenic and non-pathogenic strains has been useful in elucidating stepwise evolution of pathogenicity and the associated factors. Comparisons of pathogenic and non-pathogenic strains predicted recombination-driven species diversification and host expansion in *X. arboricola*[21]. A distinct phylogenetic cluster of non-pathogenic strains lacked the *hrpG* and *hrpX* genes essential for regulation of the T3SS (REF.[46]). Acquisition and positive selection of several pathogenicity-associated genes at different evolutionary phases were shown for *X. arboricola*[46,127]. Genetic exchange from genera other than *Xanthomonas* has also been reported. For example, a recent study found a strain of *X. arboricola* pv. *juglandis* carrying a large genomic segment (~95 kb), with genes conferring copper resistance, that resembled genes in *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa*[18]. Horizontal gene transfer resulting in exchange of virulence factors between *Xanthomonas* spp. has been reported on several occasions. Although common in several *Xanthomonas* spp., TALEs were not reported until recently in *X. perforans*. Interestingly, two TALEs — AvrHah1 and a homologue of AvrBs3, PthXp1 — occurred in distinct lineages, indicating multiple independent TALE acquisitions[22]. TALEs have been studied extensively in *X. oryzae* pathovars, which carry a large repertoire of these effectors. Strains of *X. oryzae* that had been exposed to previously domesticated rice cultivars were shown to carry higher numbers of TALEs than strains not exposed[128]. Additionally, due to the repetitive region shared among the TALEs, recombination is frequent, thus creating novel TALEs[128].

Overall, local host and environmental factors likely drive the emergence and selection of any pathogen, including *Xanthomonas* spp. Genome-wide recombination between *X. perforans* and *X. euvesicatoria* led to intraspecific variability in effector repertoires and virulence factors, with different recombinants in different global production regions[48]. The *X. perforans* strains isolated in the early 1990s in Florida, USA, carried bacteriocins that were antagonistic to the endemic *X. euvesicatoria* population[129]. By the late 1990s and 2000s, gradual erosion of bacteriocin activity was observed in *X. perforans* strains as distinct phylogenetic lineages emerged as a result of recombination with other closely related *Xanthomonas* spp.[130]. Once introduced to a new population, virulence factors can be selected for or gradually erode from the gene pool. In *X. perforans*, *avrBsT* has increased in frequency and has become established in the Florida population, whereas *avrXv3* was lost[130,131]. Similarly, distinct *X. oryzae* pv. *oryzae* lineages isolated from the Philippines and shifts in pathogenic races were correlated with change in the cultivars[132]. The apparent fitness of emerging *X. oryzae* pv. *oryzae* races was speculated to be associated with changes in cropping patterns, fertilizer use, environment and overall adaptation of the pathogen[49]. Among the 30 TALE families described in *X. oryzae* pv. *oryzae* strains isolated from the Philippines, diversification was observed only after the lineage formation and likely during host adaptation[132]. These findings illustrate the dynamics of *Xanthomonas* spp. diversity and evolution of virulence.

Plant resistance and evasion

Xanthomonas spp. stimulate PTI and ETI. Host immunity is triggered by flagellin, potentially through several PAMP receptors[133–136]. *FLS2* encodes the flagellin receptor, which recognizes the immunogenic component of flagellin[133,137]. Host glycosidases, such as β-galactosidase 1, together with host proteases, release immunogenic peptides from flagellin of plant pathogenic bacteria[137]. However, some variants of flagellin from *Xanthomonas* spp. fail to trigger an FLS2-dependent response[138]. Furthermore, flagellin from *X. oryzae* pv. *oryzae* fails to

Pathogenic races
Groups of strains that belong to the same or closely related bacterial species, characterized by differential responses (compatible or incompatible reaction) on an array of hosts.

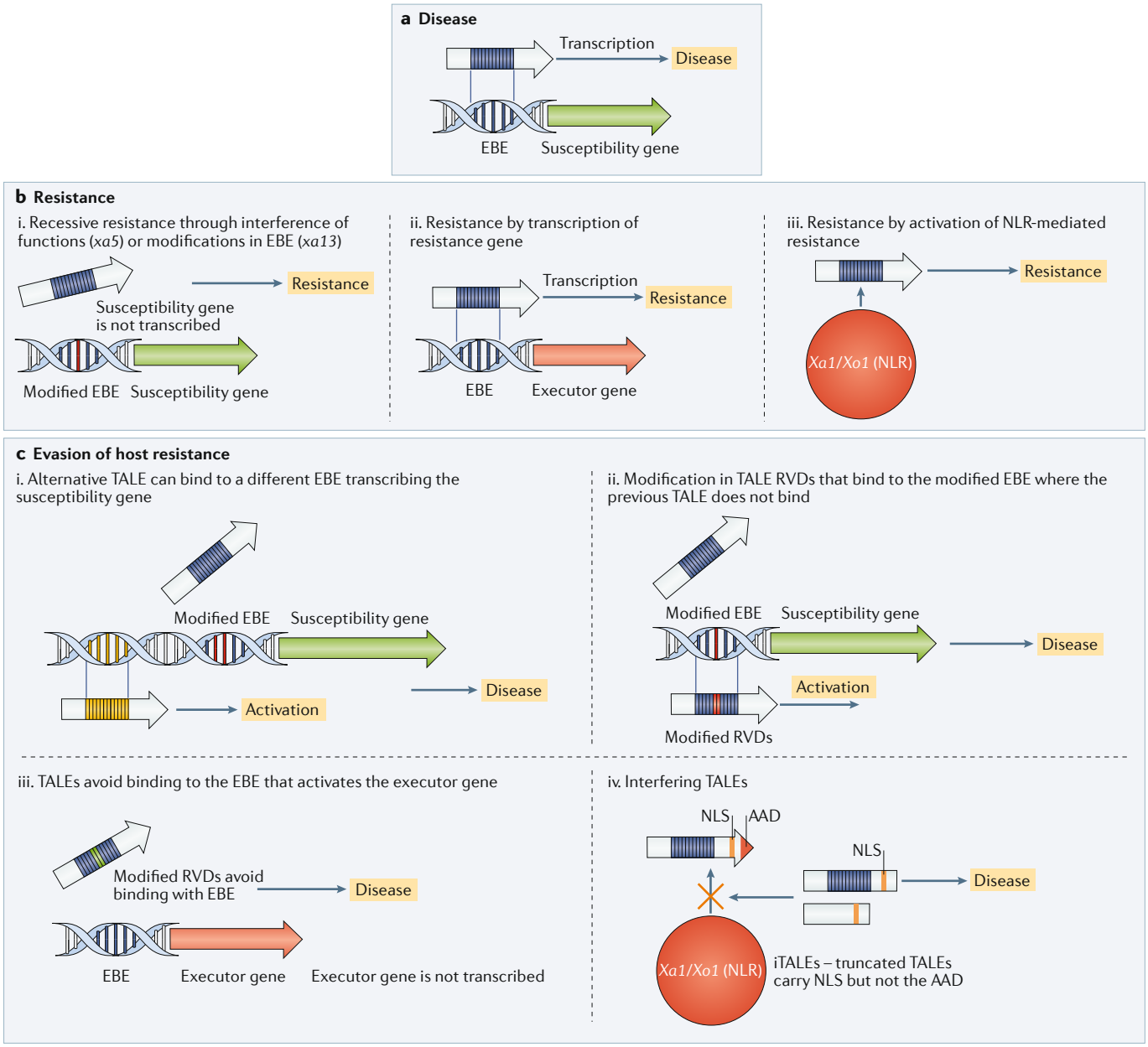


Fig. 4 | **Role of *Xanthomonas* TALEs in plant susceptibility and resistance.** **a** | The repeat regions of the transcription activation-like effectors (TALEs) bind to the effector binding elements (EBEs) in the host and transcribe a host susceptibility gene for pathogenicity[92,96]. **b** | Several mechanisms underlie host resistance: (i) a modified EBE (red) avoids TALE binding and, thus, the susceptibility gene is not transcribed, resulting in host resistance[96,98]; (ii) the host carries resistance instead of a susceptibility gene under control of the promoter targeted by the TALE — this set-up tricks the pathogen into activating transcription of a resistance gene when the TALE binds to the executor EBE, leading to host resistance[107,111,172]; and (iii) the host uses recognition by nucleotide binding, leucine-rich repeat (NLR) protein of the TALE to trigger ETI[107–109]. **c** | In response, pathogens have evolved mechanisms to evade host resistance and induce pathogenicity: (i) variable copies of TALEs can bind to different EBEs, transcribing the same susceptibility gene; (ii) *Xanthomonas* spp. can use modified repeat variable di-residues (RVDs) to bind the modified EBE and the susceptibility gene is successfully transcribed to cause disease[173]; (iii) *Xanthomonas* spp. can modify the RVDs to avoid binding to the EBE and activating the executor gene, which leads to host susceptibility[174]; and (iv) interfering TALEs (iTALEs) lack the activation domain, and thus interfere with the function of NLR proteins[109]. AAD, acidic activation domain; NLS, nuclear localization signals.

elicit an FLS2-dependent response in *A. thaliana* or a response to a rice *FLS2* homologue, whether in rice or transferred to *A. thaliana*[139]. Other bacteria also trigger host responses, including LPS, xanthan gum, peptidoglycan, cell wall-degrading enzymes, elongation factor Tu and quorum sensing molecules[140–144]. Many T3SEs of *Xanthomonas* spp. suppress PTI[72,83–85,145–149] (FIG. 3). Basal immunity has also been reported to be suppressed by other extracellular compounds, including the exopolysaccharide xanthan[144].

Dominant resistance genes, which comprise the distinct components of ETI, target many species of *Xanthomonas*. The resistance gene *Xa21* has similar functions to the receptor-like kinases involved in PTI[150].

Dominant resistance
Resistance conferred by a single dominant resistance gene in plant hosts.

XA21 is broadly effective against strains of *X. oryzae*. The receptor recognizes an extracellular, sulfated small peptide called RaxX[151]. Some other *Xanthomonas* spp. also produce RaxX[152]. RaxX can mimic plant peptide hormones and may have a function in virulence[151]. Several resistance genes are members of the NLR family, including *Bs2* (pepper), *Bs4* (tomato), *Xa1* and *Xo1* (rice), and *Zar1* (*A. thaliana*)[107,108,110,153,154]. Each of these NLRs has cognate effectors in the respective pathogens, which are subject to various evolutionary processes enabling evasion of host ETI; for example, disruption of avirulence gene expression through frameshift mutation, stop codons or transposon insertion[130,155]. Likewise, several avirulence genes are carried by *Xanthomonas* spp. on self-transmissible plasmids and may be lost over the course of a single season[156]. The durability of dominant resistance genes that recognize major pathogen virulence or fitness factors showed mixed results over the years. Disruption of AvrXa7 activity in *X. oryzae* pv. *oryzae* strains in response to *Xa7* recognition in rice resulted in the loss of avirulence activity, although the pathogen incurred a substantial fitness penalty[157]. Nevertheless, *Xa7* recognition can also be overcome by acquisition of alternate effectors with no *Xa7*-dependent ETI activity that provides a similar fitness effect[89,93,97,98]. By contrast, a single amino acid substitution in AvrBs2, which is required for full virulence of numerous *Xanthomonas* spp., enabled *X. euvesicatoria* to evade *Bs2* recognition in commercial pepper varieties, while maintaining virulence[146,149,158,159]. Some T3SEs can suppress ETI in specific cases[30]. The NLRs XA1 and XO1 are triggered by several TALEs, and therefore loss of even one or two TALEs from *X. oryzae*, which contains upwards of 27 different genes, is problematic. Furthermore, iTALEs, a class of truncated genes encoding TALEs, which were previously considered pseudogenes, can inhibit the recognition by XA1 and XO1 (REFS[109,160]) (FIG. 4).

Host resistance can also occur as recessive resistance. Pepper contains *bs5* and *bs6*, which confer resistance to *X. euvesicatoria*[161,162]. Soybean contains the recessive resistance gene *rxp*, which provides broad resistance against strains of *X. axonopodis* pv. *glycines*[163]. TALE-mediated susceptibility is especially prone to recessive resistance due to DNA polymorphisms that prevent TALE binding to specific DNA sequences[89,98,164] (FIG. 4). TALEs function through the transcriptional activation of plant susceptibility genes, which in rice and citrus they are crucial for effective host invasion[92]. The recessive resistance gene *xa5* interferes with TALE function and evasion occurs through strong induction of *OsSWEET11* or *OsSWEET14*, indicating that compatibility depends on expression levels rather than on activation of a specific susceptibility gene[165]. Recessive resistance in rice that happens due to polymorphism in the promoters of susceptibility genes can be evaded by TALEs with alternative binding sites[97,98].

Non-conventional approaches

Understanding of *Xanthomonas*–host interactions has fuelled the development of disease-resistant hosts through genetic modifications. A notable example is the elongation factor-TU receptor (EFR) in *A. thaliana*, which recognizes a conserved EF-Tu domain in most bacterial genera[134]. Transfer of *AtEFR* from *A. thaliana* to tomato reduced the severity of bacterial spot disease caused by *X. perforans* in field conditions[166]. A second example relates to TALEs that bind specific DNA sequences (effector binding elements (EBEs)). Modifying EBEs so that TALEs can no longer bind can be an effective method for developing resistance. CRISPR–Cas9-mediated citrus canker resistance has been developed in grapefruit and sweet orange through modifications in the effector binding promoter region of *CsLOB1*[106,167]. A similar approach has been used to modify three *SWEET* genes targeted by TALEs from *X. oryzae* pv. *oryzae* in rice. EBEs targeted by *avrXa7* and *pthXo3* were modified in rice using TALE nucleases (TALENs), leading to the loss of susceptibility gene expression and resistance against *X. oryzae* pv. *oryzae* strains carrying the two genes[168,169]. Alternatively, EBEs can be added to the promoters of the resistance genes, which leads to the activation of resistance in the presence of TALEs. Researchers introduced 14 EBEs that match distinct *X. citri* TALEs into the $ProBs3_{14EBE}$ promoter and fused it to the avirulence gene *avrGf1*, which induces a hypersensitive response in grapefruit and sweet orange[170]. Using resistance genes from closely related species to target single genes could lead to rapid development of pathogen virulence. Durability and a combination of multiple resistance genes targeting several pathogenicity factors should be considered when developing host resistance.

Conclusions

Xanthomonas spp. use a multitude of virulence factors that interfere with host cellular pathways. Recent studies on *Xanthomonas*–host interactions have been vital for unlocking mechanisms associated with *Xanthomonas* spp. pathogenicity, diversity and host specificity. The T3SS and associated Xop effectors are major factors influencing pathogenicity and virulence. Studies have further evaluated the importance of other pathogenicity factors, including T2SS, small RNAs and others. With an improved understanding of dynamics of virulence factors in pathogen populations, we will have a better understanding of *Xanthomonas* evolution in relation to host/tissue specificity and expansion. Research has evolved to integrate these novel findings when developing host resistance against *Xanthomonas* spp. Collectively, *Xanthomonas* spp. have been a model system to understand emerging bacterial plant pathogens and diversity.

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