Review

Bacterial pathogenesis of plants: future challenges from a microbial perspective

Challenges in Bacterial Molecular Plant Pathology

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SUMMARY

FUTURE CHALLENGES IN PLANT PATHOLOGY:

Plant infection is a complicated process. On encountering a plant, pathogenic microorganisms must first adapt to life on the epiphytic surface, and survive long enough to initiate an infection. Responsiveness to the environment is critical throughout infection, with intracellular and community-level signal transduction pathways integrating environmental signals and triggering appropriate responses in the bacterial population. Ultimately, phytopathogens must migrate from the epiphytic surface into the plant tissue using motility and chemotaxis pathways. This migration is coupled with overcoming the physical and chemical barriers to entry into the plant apoplast. Once inside the plant, bacteria use an array of secretion systems to release phytotoxins and protein effectors that fulfil diverse pathogenic functions (Fig. 1) (Melotto and Kunkel, 2013; Phan Tran et al., 2011).

As our understanding of the pathways and mechanisms underpinning plant pathogenicity increases, a number of central research challenges are emerging that will profoundly shape the direction of research in the future.

- We need to understand the bacterial phenotypes that promote epiphytic survival and surface adaptation in pathogenic bacteria. How do these pathways function in the context of the plant-associated microbiome, and what impact does this complex microbial community have on the onset and severity of plant infections?
- The huge importance of bacterial signal transduction to every stage of plant infection is becoming increasingly clear. However, there is a great deal to learn about how these signalling pathways function in phytopathogenic bacteria, and the contribution they make to various aspects of plant pathogenicity.
- We are increasingly able to explore the structural and functional diversity of small-molecule natural products from plant pathogens. We need to acquire a much better understanding of the production, deployment, functional redundancy and physiological roles of these molecules.

Type III secretion systems (T3SSs) are important and well-studied contributors to bacterial disease. Several key unanswered questions will shape future investigations of these systems.

- We need to define the mechanism of hierarchical and temporal control of effector secretion.
- For successful infection, effectors need to interact with host components to exert their function. Advanced biochemical, proteomic and cell biological techniques will enable us to study the function of effectors inside the host cell in more detail and on a broader scale.
- Population genomics analyses provide insight into evolutionary adaptation processes of phytopathogens. The determination of the diversity and distribution of type III effectors (T3Es) and other virulence genes within and across pathogenic species, pathovars and strains will allow us to understand how pathogens adapt to specific hosts, the evolutionary pathways available to them, and the possible future directions of the evolutionary arms race between effectors and molecular plant targets.

Although pathogenic bacteria employ a host of different virulence and proliferation strategies, as a result of the space constraints, this review focuses mainly on the hemibiotrophic pathogens. We discuss the process of plant infection from the perspective of these important phytopathogens, and highlight new approaches to address the outstanding challenges in this important and fast-moving field.

Keywords: bacterial signalling, phytotoxins, plant infection, plant pathogenicity, Pseudomonas, type III effectors, Xanthomonas.

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INTRODUCTION—SURVIVING STRESS ON THE PLANT EPIPHYTIC SURFACE

The surface of plant leaves is a harsh environment for pathogenic microorganisms, with bacteria regularly exposed to desiccation, ultraviolet irradiation, adverse temperature changes and mechanical disruption, for example, by strong winds. Despite these challenges, epiphytic bacteria often reach population densities of between $10^6$ and $10^7$ cells/cm² of leaf (Andrews and Harris, 2000). Although indirect, microbial persistence on the plant surface may be considered as a virulence strategy, as plant disease symptoms are effectively reduced by the disruption of pathways involved in epiphytic survival. Furthermore, surface survival pathways may also play important roles during other stages of infection.

Metabolic responses to cold and osmotic shock, and desiccation, stress materially contribute to epiphytic survival (Djonovic et al., 2013; Freeman et al., 2013; Wu et al., 2012). For example, the osmoprotectant trehalose has been implicated in Pseudomonas syringae survival and population maintenance in the phyllosphere (Freeman et al., 2010), and has also been linked to plant pathogenicity in the opportunist Pseudomonas aeruginosa, where it has been suggested to promote the acquisition of nitrogen, enhancing proliferation in the leaf apoplast (Djonovic et al., 2013). Exopolysaccharide (EPS) production plays an important role in the epiphytic survival of the plant-associated microbiome, including pathogenic Xanthomonas spp. (Dunger et al., 2007) and P. syringae (Yu et al., 1999). Different EPS molecules have been shown to contribute to plant surface colonization and infection, including the well-studied polymers xanthan (Dunger et al., 2007), levan and alginate (Freeman et al., 2013; Laue et al., 2006; Yu et al., 2013), although a large number of novel EPSs undoubtedly exist in nature whose role in plant pathogenicity remains to be determined. For example, the acetylated cellulose Wss is important for effective root colonization by the commensal bacterium Pseudomonas fluorescens (Gal et al., 2003), and is also present in P. syringae pv. tomato (Pto) DC3000 (Prada-Ramirez et al., 2016). The aggressive kiwi pathogen P. syringae pv. actinidiae (Psa) NZ V-13 produces two novel, previously uncharacterized, polysaccharides: a branched α-1,3-D-rhamnan and an α-1,4-linked glucan polymer (Ghods et al., 2015). The genomes of P. syringae pathovars also encode a number of EPS loci that have been characterized in other Pseudomonas species, but whose contribution to plant infection is undefined. These include the widespread pel and psl operons (Winsor et al., 2011), which play important roles in persistence during cystic fibrosis lung infections by the opportunistic pathogen P. aeruginosa (Malone, 2015).

EPS molecules are associated with a number of different plant interaction functions, most of which are linked to epiphytic survival, contributing to freeze–thaw resistance (Wu et al., 2012), desiccation and osmotic stress tolerance (Chang et al., 2007; Freeman et al., 2013), and maintenance of the microbial population (Dunger et al., 2007). In addition to their roles in protecting bacteria on the plant surface, EPS molecules from several phytopathogens have also been implicated in immune evasion, possibly mediated by the quenching of calcium signalling during the plant immune response (Aslam et al., 2008), whereas the levan biosynthetic locus in P. syringae pv. syringae (Pss) B728a is strongly upregulated in the apoplast relative to the leaf surface, suggesting a further, post-infection role in virulence (Yu et al., 2013). Laue et al. (2006) have suggested that accumulated levan may function as a nutrient storage source to enable later stages of biofilm formation, although it remains to be determined whether levan fulfils a similar role during plant infections. Finally, Psa NZ V-13 forms biofilms on both external and internal plant surfaces at different

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**Fig. 1** The process of plant infection. 1, Surface survival and biofilm formation. 2, Flagella/pili-driven migration across plant surfaces to apoplastic entry sites. 3, Release of phytotoxins to bypass stomatal closure. 4, Ice nucleation to damage plant surfaces. 5, Extracellular enzyme secretion to degrade cell walls and damage plant tissue. 6, Phytotoxin secretion to modify plant physiology, metabolism and immune responses.
stages of infection, again consistent with an apoplastic role for phytopathogenic EPS (Renzi et al., 2012).

**SIGNAL TRANSDUCTION IN PLANT PATHOGENS**

Life on plant leaves is both complex and dynamic, with epiphytic pathogens continually deciding whether to express traits that enable them to survive on the surface, or to bypass the leaf epidermis and enter the apoplast, before propagating in the intercellular space or colonizing the plant vascular system. This decision-making process is a critical factor in the onset, virulence and persistence of plant infections, and is mediated, to a large degree, by small-molecule-based signalling pathways. These systems regulate motility and biofilm formation, the expression of virulence genes and communication with other bacteria, both within and between species. In plant pathogens, a large number of signalling systems have been described, with the main ones being quorum sensing (QS) and second messenger pathways, such as cyclic guanosine 3’,5’-monophosphate (cGMP) signalling. Here, we focus our attention on signalling in Xanthomonas spp. and *P. syringae*. These economically important, model phytopathogens use different, well-characterized signalling systems: Xanthomonas campestris pv. campestris (Xcc) produces a fatty acid QS signal called diffusible signal factor (DSF), which is linked to cGMP signalling. QS in *Pss*, however, is mediated by the acyl-homoserine lactones (AHLs), a widely distributed QS signal among Gram-negative bacteria. Although there is a body of work on cGMP signalling in *Xanthomonas* (see below), the role of cGMP signalling in *P. syringae* pathogenesis has only been described recently (Engl et al., 2014; Perez-Mendoza et al., 2014; Pfeilmeier et al., 2016).

**QS IN PLANT PATHOGENS**

Bacteria use QS to communicate and assess cell density by producing small signalling molecules, called auto-inducers, which are sensed by bacteria in the immediate environment and affect gene expression once a threshold level reflecting a critical cell density is attained. A large number of QS molecules have been described, with various roles and high structural diversity (Fig. 2). In plant-pathogenic bacteria, the major characterized signals are AHLs, DSF and Ax21, which are discussed in more detail below.

AHLs represent a major class of QS molecules in plant pathogens. Most AHL systems consist of two genes, coding for an AHL receptor and an AHL synthase. Transcription of the synthase gene is positively regulated by the AHL itself. *Pseudomonas syringae* produces the AHL molecule 3-oxo-C6-HSL (HSL, homoserine lactone) (Quinones et al., 2004). The *ahlR/ahhR* system in *Pss* regulates motility and EPS production, essential factors for plant colonization and virulence (Quinones et al., 2005). The crown gall pathogen *Agrobacterium tumefaciens* produces 3-oxo-C8-HSL to control virulence through the stimulation of Ti plasmid copy numbers, whereas, in the rice pathogen *Burkholderia glumae*, the production of C8-HSL regulates phytotoxin secretion and motility through control of flagellum assembly (Kim et al., 2004; Pappas and Winans, 2003). Some bacteria produce several AHLs. This is the case for *P. aeruginosa*, which uses three AHL pathways (LasI/LasR, RhlR-RhlR and the orphan regulator QsrR) to produce 3-oxo-C12-HSL and C4-HSL, which affect the expression of more than 300 genes, many involved in toxin secretion, motility and biofilm formation (Schuster et al., 2003). *Pseudomonas aeruginosa* also produces a third QS molecule, 2-heptyl-3-hydroxy-4-quinolone (also called PQS for *Pseudomonas* quinolone signal), involved in the production of virulence factors and the control of biofilm formation (Allesen-Holm et al., 2006; Pesci et al., 1999). The expression of lasB, which encodes a type II secreted protease, has been shown to be mediated by the las, rhl and PQS systems in *P. aeruginosa*, whereas PQS synthesis is also controlled by the two AHL signals, highlighting the complexity of gene regulation through QS signalling systems (Pesci et al., 1999). Thus far, PQS-like signals have not been detected in other phytopathogenic pseudomonads.

Another QS signal of interest is the recently described protein Ax21. Ax21 is a small sulfated protein produced by the rice pathogen *Xanthomonas oryzae* pv. oryzae, which acts as a QS molecule required for virulence gene expression (Han et al., 2011). Similar proteins have subsequently been identified in other pathogens, including *X. oryzae* pv. oryzae (Qian et al., 2013) and the commensal *Stenotrophomonas maltophilia* (McCarthy et al., 2011), and have been shown to be essential for virulence, biofilm formation, motility and EPS production. However, the Ax21 homologue in *Xylella fastidiosa* was shown not to be required for virulence or biofilm formation, supporting the species-specific evolution of QS signal-mediated regulation of virulence (Pierce et al., 2014).

*Xanthomonas* species use DSF, which is a different class of QS molecule composed of unsaturated fatty acids. The DSF signal sensed by Xcc, a pathovar that causes black rot in crucifers, is cis-11-methyl-dodecenoic acid (Wang et al., 2004) (Fig. 2) and is required for virulence and to regulate xanthan and protease synthesis (Barber et al., 1997; Tang et al., 1991). Since its original description, the DSF family has expanded, with new molecules having a similar unsaturated fatty acid backbone, but showing some variation in structure. For example, *X. fastidiosa* DSF is cis-2-tetradecenoic acid (Beaulieu et al., 2013). Depending on the growth conditions, *X. oryzae* pv. oryzae produces DSF and two other molecules: cis-2-dodecenoic acid (similar to the signal produced by *Burkholderia cenocepacia*, BDSF) and cis,cis-11-methyl-dodeca-2,5-dienoic acid (CDSF) (Fig. 2) (He et al., 2010). DSF signalling is mediated by products of the rpf (regulation of pathogenicity factor) gene cluster in Xcc, the mutation of which leads to reduced virulence (Barber et al., 1997). DSF is synthesized by the putative enoyl-CoA hydratase RpfF, whereas the fatty acyl-CoA ligase RpfB has been implicated in signal turnover (Barber et al., 1997; Bi et al., 2014; Zhou et al., 2015). It is then sensed by
RpfC, a hybrid sensor kinase, which also negatively regulates DSF synthesis (Slater et al., 2000). RpfC is part of a two-component system with the response regulator RpfG. RpfG consists of a phosphorylatable receiver domain and an HD-GYP domain, with phosphodiesterase (PDE) activity involved in cdG degradation (Slater et al., 2000). On signal transduction through the RpfC/RpfG system, the expression of virulence genes is activated by cdG degradation and release of Clp (for Crp-like protein) transcription regulator repression (Fig. 3) (Chin et al., 2010; Ryan et al., 2006). In addition to RpfC, a further DSF sensor, RpfS, has been described recently in Xcc and has been shown to be required for virulence to Chinese radish (An et al., 2014a). It is thus very likely that other DSF sensors still remain to be discovered.

Similar systems have been described in other plant pathogens, such as Xy. fastidiosa and additional Xanthomonas species, with some notable differences in regulatory outcomes. For example, in Xy. fastidiosa, rpfF mutation leads to increased virulence and increased colonization of grape xylem vessels (Chatterjee et al., 2008b; Newman et al., 2004). Moreover, a recent study of Xy. fastidiosa RpfF has shown that this DSF synthesis protein is also essential for signal transduction (Ionescu et al., 2013). Xylella fastidiosa apparently uses DSF signalling in a multifaceted manner to regulate its virulence, adhesion and colonization potential, and depending on whether it is infecting a plant or an insect (Chatterjee et al., 2008a,a).

Although the rpf gene cluster appears to be conserved across X. campestris pathovars, differences exist between the model species Xcc and other species within the Xanthomonas genus. Depending on the species, the pathovar or even the strain, different phenotypes can be observed when mutating genes in the rpf cluster. For example, in X. oryzae pv. oryzae, RpfC has been shown to be required for virulence, but rpfC mutation has no effect on protease production (Tang et al., 1996). These differences are probably a result of the use of different cdG binding targets for gene regulation (see below) and further highlight the remarkable adaptive potential of plant pathogens.

**cdG SIGNALLING**

Second messenger nucleotides, such as cyclic adenosine monophosphate (cAMP) and cdG, are known to function as signalling molecules and have been shown to play numerous roles in the control of bacterial virulence. We limit our discussion of this complex field to the role of cdG in plant pathogenesis. As discussed above, QS and DSF signalling are closely linked to cdG signalling in plant pathogens and constitute additional layers of virulence...
regulation. cdG is a second messenger found in most bacteria and is involved in the regulation of a wide range of functions, including virulence, biofilm formation and motility. cdG was initially described in Acetobacter xylinum as a regulator of cellulose synthesis (Ross et al., 1987). Since its discovery, there has been a great deal of research into the synthesis of cdG, the proteins involved in signal transduction and its binding targets (for a detailed recent review on cdG, see Romling et al. (2013)]. Intracellular cdG levels are controlled by diguanylate cyclases (DGCs) and PDEs. DGCs, containing a GGDEF domain, synthesize cdG from two guanosine triphosphate (GTP) molecules, and PDEs, with an EAL or an HD-GYP domain, degrade the second messenger. The activity of these proteins is controlled by environmental cues, thus enabling bacteria to modulate their cdG levels to respond to the environment. For this reason, DGC/PDEs often contain additional domains involved in signalling (GAF, PAS or REC domains, for example). In general, high levels of cdG promote a sessile lifestyle and biofilm formation and inhibit virulence and motility. The second messenger exerts its action by interacting with a diverse array of binding domains, including proteins with a PilZ domain, enzymatically inactive DGCs and PDEs, RNA riboswitches and transcriptional regulators, such as Clp and the Pseudomonas motility/ EPS regulator FleQ (Romling et al., 2013).

In the DSF/rpf system, cdG interacts with Clp, preventing Clp promoter binding and thus the transcription of its target genes (Fig. 3). In Xcc, Clp has been shown to control the expression of genes encoding extracellular enzymes (Chin et al., 2010). The same interaction between Clp and cdG has been observed in Xanthomonas axonopodis pv. citri (Leduc and Roberts, 2009). Another two-component system, RavS/RavR, required for virulence and linked to cdG, has been found in Xcc. The RavR protein has been shown to alter cdG levels through the PDE activity of its EAL domain and to regulate virulence factor expression via the transcriptional regulator Clp (He et al., 2009). This signal in this case is not DSF, but the sensing of hypoxia through the PAS domains of RavS (He et al., 2009). This signalling system appears to work synergistically with RpfC/RpfG and DSF signalling to ensure survival and pathogenicity in plant xylems, where low oxygen conditions are found.

The HD-GYP PDE domain contains two conserved motifs separated by a small, less conserved region: the HD residues, which are required for enzymatic activity, and the GYP residues, which are involved in protein–protein interactions with GGDEF domains (Ryan et al., 2006, 2010). Mutation of either residue of the RpfG HD motif results in reduced extracellular enzyme production, and alanine substitutions of the GYP residues lead to reduced motility (Ryan et al., 2006, 2010). With both motifs playing a role in the regulation of virulence in Xcc, RpfG exerts its regulatory action through the mediation of cdG levels, but also by direct interaction with other proteins involved in the modulation of cdG levels (Fig. 3).

The role of cdG signalling in plant-pathogenic Pseudomonas has only recently been addressed. cdG-regulated systems control multiple important aspects of phytopathogenic Pseudomonas behaviour, and affect the virulence of various species and pathogens (Engl et al., 2014; Perez-Mendoza et al., 2014; Pfeilmeier et al., 2016). A specific role of cdG has been suggested in the regulation of flagellum and T3SS activity (Trampari et al., 2015), and the control of proteome composition through ribosomal modification (Little et al., 2016). The Gac-Rsm network, which controls secretion systems, biofilm formation and QS, as well as siderophore and toxin production (Vakulskas et al., 2015), affects cdG levels by manipulating PDE/DGC production (Moscoso et al., 2014, 2011). GacA has also been linked to T6SS expression in P. syringae (Records and Gross, 2010), whereas in trans overexpression of the RNA-binding post-transcriptional regulator RsmA represses the production of numerous secreted virulence factors (Kong et al., 2012). cdG signalling in plant pathogenesis has been mainly studied in certain model Xanthomonas and Pseudomonas species, but its regulatory roles in other important phytopathogens, such asRalstonia solanacearum, are poorly understood.

Fig. 3 The Rpf (regulation of pathogenicity factor) hybrid quorum sensing/cyclic di-guanosine monophosphate (QS/cdG) signalling system in Xanthomonas spp. On sensing the diffusible QS signal diffusible signal factor (DSF) (produced by the synthase RpfF), the sensor kinase RpfC phosphorylates and activates the HD-GYP phosphodiesterase (PDE) RpfG. The PDE activity of RpfG degrades cdG, leading to a reduction in biofilm formation and release of Clp (for Clp-like protein) repression. This, in turn, leads to Clp-mediated virulence gene transcription. RpfG also interacts with several GGDEF domain-containing proteins, which, in turn, regulate bacterial motility.

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(Genin and Denny, 2012). Members of the genera *Serratia* and *Erwinia* have also been shown to use cdG signalling during plant infection (Romling et al., 2013), although, once again, the fine details of the signalling networks in each case remain poorly defined. The complex environments of the soil and the phyllosphere seem to promote correspondingly complex regulatory networks to adapt and respond to environmental signals. *Xcc* contains over 30 genes coding for DGCs and PDEs (Ryan et al., 2006), and this species is by no means unusual among the phytopathogens. Moreover, the list of experimentally verified cdG-binding proteins continues to expand, with the discovery of new cdG-binding targets, such as the YajQ family protein XC_3703 in *Xcc* (An et al., 2014b) and the type III injectisome ATPase HrcN in *P. syringae* (Trampari et al., 2015).

Plant-pathogenic bacteria display evolved cell–cell signalling systems with multiple, simultaneous and overlapping input signals, non-linear gene regulation and extensive crosstalk between signalling systems and between different bacterial species on the plant surface. It is therefore very difficult to effectively define individual signalling pathways, or to assign discrete functions to these pathways during infection. Nonetheless, as our understanding of intracellular signalling increases and additional experimental and analytical tools become available, the critical role of bacterial signalling pathways to plant pathogenesis will undoubtedly become much clearer.

**ADAPTING TO THE PLANT ENVIRONMENT**

Downstream of the signalling pathways that integrate pathogen responses to the environment are transcriptional changes associated with life on plant surfaces. Marco et al. (2005) have shown that genes involved in virulence, membrane transport, nutrient acquisition and intracellular signalling are up-regulated in *Pss* B728a on the surface of bean plants. A subsequent microarray analysis of *Pss* B728a isolated from different leaf sites (Yu et al., 2013) showed that epiphytic locations specifically favour flagellar motility and the production of surfactants to facilitate surface swarming, chemosensing and chemotaxis, suggesting active microbial relocation across the leaf surface. Increased expression of phenylalanine degradation loci was also observed, possibly as a countermeasure to phenylpropanoid-based plant defences. In contrast, aposporal growth was linked to the expression of γ-aminobutyric acid (GABA) metabolic genes, antioxidant production and the biosynthesis of secondary metabolites, for example, the lipodepsipeptide phytotoxins and syringolin A (Yu et al., 2013).

A further study by Freeman et al. (2013) examined differences in transcriptional responses between two *P. syringae* strains, *Pss* B728a and *Pto* DC3000, on osmotic shock, and used these data to explain the observed differences in epiphytic survival between the two strains. Consistent with its higher survival rate on the leaf surface, *Pss* B728a exhibited increased osmotolerance relative to *Pto* DC3000, and higher rates of uptake of plant-derived osmoprotectants. The two strains also displayed markedly distinct transcriptional responses to osmotic shock; multiple loci were up-regulated in *Pss* B728a, including type VI secretion system (T6SS) and alginate biosynthesis pathways, whereas *Pto* DC3000 showed no change or repression of these loci and down-regulated expression of T3SS genes (Freeman et al., 2013). Based on this, Freeman et al. (2013) suggested that epiphytic competence was mediated by strong, actively induced osmotolerance, accompanied by the synthesis of alginate and the T6SS.

A particularly well-studied example of a plant environment-sensing regulatory pathway is the induction of virulence gene expression in *Ag. tumefaciens* by low pH, plant-associated sugars and phenolics, such as acetosyringone (Lee et al., 1995; Peng et al., 1998). Expression of the vir regulon is controlled by the two-component VirAG regulatory system, consisting of a homodimeric transmembrane histidine protein kinase (VirA) which directly binds to acetosyringone and other target phenolic molecules (Lee et al., 1995), and controls the transcriptional regulator VirG (Wise and Binns, 2015). Sugar moieties are sensed by the accessory periplasmic glucose–galactose-binding protein ChvE, which interacts directly with VirA and enhances its sensitivity to phenolics (Peng et al., 1998). The VirAG/ChvE pathway enables *Agrobacterium* to specifically induce virulence genes in response to specific chemical signals synthesized at plant wound sites, and so to effectively induce the formation of crown gall tumours.

The examination of individual pathogen interactions in defined model plant systems has yielded an enormous amount of valuable information on the pathways that govern plant surface association and infection. However, these studies are, by necessity, highly simplified models for phytopathogen interactions with crops in the field. In a natural environment, pathogenic behaviour is extensively modulated by interactions with other constituents of the plant microbiome, with both synergistic and antagonistic interactions markedly affecting epiphytic survival and plant infection (Andrews and Harris, 2000; Delmotte et al., 2009; Ritpitakphong et al., 2016). An understanding of pathogen infection and plant surface colonization in the context of the whole microbiome represents a huge and challenging new frontier in plant pathogenicity research. To address this challenge, recent advances in sequencing and the development of additional, advanced techniques for the interrogation of bacterial populations are starting to be used to shed light on the distribution and behaviour of whole populations of bacteria, both pathogenic and commensal, on plant surfaces. Sequencing-led techniques now enable us to analyse the composition of the leaf surface-associated microbiome and to determine its impacts on pathogenicity (Ritpitakphong et al., 2016), whereas metatranscriptomic (Molina et al., 2012) and proteomic analysis (Delmotte et al., 2009) techniques may be used to examine the
relevant bacterial systems for epiphytic life at the population level, and to map the regulatory transition from surface survival to migration into the apoplast. Finally, quantitative metabolomics techniques, such as imaging mass spectroscopy and quantitative nuclear magnetic resonance (Ryffel et al., 2015), have been used to analyse the in situ distribution of small molecules and metabolites in the leaf surface microbiome, and how these change on pathogen infection.

**MIGRATING ACROSS LEAF SURFACES AND INTO THE APOPLAST**

Many pathogenic bacteria respond to leaf surface contact by expressing traits that promote survival until environmental conditions allow the penetration into the apoplast. Once such conditions arise, or immediately in the case of some poor epiphytes, pathogens deploy various motility systems to allow them to migrate across the leaf surface to stomata, wounds and other access sites to the interior of the plant. Flagella-driven motility confers epiphytic fitness advantages in *P. syringae* (Haefele and Lindow, 1987), and is highly important for surface colonization and effective plant virulence (Tans-Kersten et al., 2001). Flagella gene expression is typically coordinated with chemotaxis (Yu et al., 2013) and the production and deployment of surfactant molecules that enable the bacteria to swarm across solid surfaces, such as leaves (Burch et al., 2012). The importance of flagellar motility primarily during the early stages of infection is supported by infection data for both the soil-borne pathogen *R. solanacearum* (Tans-Kersten et al., 2001) and *P. syringae* (Pfeilmeier et al., 2016). In both studies, suppression of flagella biosynthesis, or deletion of flagella genes, led to significantly compromised plant infections, but only when bacteria were sprayed/soaked onto intact plants. When bacteria were directly applied to cut petioles (Tans-Kersten et al., 2001), or when the requirement for apoplastic penetration was bypassed by syringe infiltration of bacteria (Pfeilmeier et al., 2016), no loss of virulence was seen in either case. Interestingly, the deletion of several signalling genes (*P. syringae* rimK (Little et al., 2016), ppiS from *Xcc* (An et al., 2014a) and *xbmR* from *X. citri* ssp. *citri* (Yaryura et al., 2015)) produces similar, conditional effects, with virulence defects only seen on bacterial application to leaf surfaces. In addition to flagella, type IV pili are required for bacterial biofilm formation and attachment, twitching motility and virulence in pathogens, including *P. syringae* (Nguyen et al., 2012) and *R. solanacearum* (Kang et al., 2002).

The importance of motility during plant infection is well established. However, it is equally important that flagella production is tightly controlled, both to enable the efficient and directional migration of bacteria into the apoplast and to avoid detection by plasma membrane-localized pattern recognition receptors (PRRs), which initiate defence responses collectively referred to as PRR- or pattern-triggered immunity (PTI) (Macho and Zipfel, 2014). For this reason, flagella are under tight regulatory control, at both the level of production and expression, and through the degradation of excess flagellin monomers by the alkaline protease AprA (Pel et al., 2014). The control of motility and the regulation of the expression of flagella/pili/surfactant loci at appropriate times during plant infection are increasingly being recognized as critical components of plant pathogenicity. How these processes function in planta is currently not fully understood, and is the focus of increasing investigation.

Alongside more conventional genetics-led approaches, future research towards a full understanding of motility control and flagella gene expression during plant infection is likely to use increasingly sophisticated fluorescence microscopy and other visualization methods to examine pathogen spread in planta, together with emerging technologies that harness the increasing power and accessibility of next-generation sequencing. An exciting new example of this is Sequence Tag-based Analysis of Microbial Populations (STAMP) (Abel et al., 2015), which uses libraries of heterologously tagged microbes to quantify population bottlenecks and to infer founding population sizes for a given infection. Although STAMP has so far been mainly used for the analysis of intestinal infections with human pathogens, the technique could easily be adapted to examine the population dynamics of phytopathogen infection and dissemination in plant tissue. In principle, this technique could also be used to conduct simultaneous assays for competitive in planta infection by multiple pathogens/mutant strains, and has the potential to substantially increase the resolution and accuracy of research into the bacterial pathways that mediate plant infections.

**GETTING INSIDE THE PLANT—STOMATAL HIJACKING AND CELL WALL-DAMAGING ENZYMES**

To initiate an infection, free-living bacterial pathogens must overcome the plant’s surface defences and gain entry into the apoplast. One obvious entry point for bacteria is the stomata. However, plants close their stomatal pores as part of their innate immune response, preventing bacterial ingress (Gudesblat et al., 2009a; Melotto et al., 2006). In response, many phytopathogens, for example *Xcc* (Gudesblat et al., 2009b) and numerous *P. syringae* pathovars (Melotto et al., 2006), produce and secrete phytoxins that overcome stomatal immunity. A number of different pathogen-secreted molecules act as antistomate defence factors, including the well-studied toxins coronatine and syringolin A (Melotto and Kunkel, 2013; Zeng and He, 2010). In each case, these phytoxins function by interfering with NPR1-dependent salicylic acid (SA) signalling (Xin and He, 2013).

In addition to entry through the stomata, phytopathogens can enter the apoplast through wounds on the plant epidermis. To
this end, many pathogens produce enzymes and other proteins that damage the cell wall and thus allow entry into the plant tissue. Several pathovars of *P. syringae* (Gaignard and Luissetti, 1993) produce ice nucleating agents (INAs), and INA loci have also been detected in other pathogenic species, such as *X. campestris* (Gurian-Sherman and Lindow, 1993) and *Pantoea ananatis* (Sauer et al., 2014). The size of the protein, its membrane association and tendency to aggregate in vitro have so far confounded efforts to produce a protein structure for a bacterial INA (Garnham et al., 2011), and our understanding of their structure and function is largely based on in silico analysis. These models propose that INAs order water molecules into an ice-like clathrin lattice that raises the temperature at which ice nucleates, causing water to freeze at high sub-zero temperatures (Garnham et al., 2011). Frost damage caused by induced ice nucleation allows pathogen entry into plant tissue. Genes conferring freeze–thaw resistance have also been shown to be widespread among epiphytic bacteria (Wu et al., 2012), which would contribute to the survival of these microbes under both abiotic and pathogen-induced frost conditions, and is consistent with the idea that directed frost damage is a common trait among phytopathogens.

In contrast with the epiphytic lifestyle of *Xanthomonas* and *Pseudomonas* spp. at the beginning of an infection, other bacterial phytopathogens, such as *Xy. fastidiosa* (Chatterjee et al., 2008a) and *Phytoplasma* (Sugio and Hogenhout, 2012), use sap-feeding insects as vectors for the penetration of host tissue and as a dissemination mechanism. Phytopathogens also release numerous enzymes, mainly through type II secretion systems (Korotkov et al., 2014), which break down plant cell wall structural molecules and hydrolyse the connective lamellae between individual plant cells, providing a carbon source for the pathogen, as well as a further mechanism for apoplastic propagation and spread through the plant tissues. A good example of this latter function is the degradation of intervessel pit membranes in grapevine xylem by *Xy. fastidiosa* (Chatterjee et al., 2008a) and *Phytoplasma* (Sugio and Hogenhout, 2012), which break down plant cell wall structural molecules and hydrolyse the connective lamellae between individual plant cells, providing a carbon source for the pathogen, as well as a further mechanism for apoplastic propagation and spread through the plant tissues. A good example of this latter function is the degradation of intervessel pit membranes in grapevine xylem by *Xy. fastidiosa*, allowing systemic expansion by the pathogen (Sun et al., 2011). Extracellular degradative enzymes are structurally and functionally diverse, and include proteases, cellulases, pectinases and xylanases (Dejean et al., 2013; Lee et al., 2014; Toth et al., 2003). These cell wall-degrading enzymes are particularly associated with *Phytoplasma*, *Xylella* and *Xanthomonas* spp., and soft-rot pathogens from the genera *Erwinia* and *Pectobacterium*. These bacteria all secrete complex mixtures of enzymes and contain multiple functionally redundant isoenzymes, which has so far presented difficulties in understanding the exact contribution of individual enzymes to pathogenesis (Toth et al., 2003).

**PLANT MANIPULATION BY PATHOGENIC BACTERIA**

In addition to degradative enzymes, phytopathogenic microbes produce small, secreted phytotoxins that contribute to bacterial virulence by subverting host defence responses and increasing chlorosis and tissue necrosis. Some phytotoxins cause direct damage to plant cells, whereas others modulate metabolic and signaling pathways in the host to benefit the invading pathogen. Lipodepsipeptide toxins, such as the syringomycins and syringopeptins, are examples of the former. They are produced by non-ribosomal peptide synthetases during *P. syringae* plant infections, and are amphipathic in nature, inducing tissue necrosis by forming pores in the plasma membranes of plant cells (Melotto and Kunkel, 2013). The modified peptide toxins phaseolotoxin, mangoxin and tabtoxin are produced by various *P. syringae* pathovars, and induce chlorosis and necrosis in plant tissue. These toxins target the enzymes involved in amino acid biosynthesis, inhibiting their activity and interfering with nitrogen metabolism. The resulting accumulation of nitrogen-containing intermediates can be utilized by the pathogen as a food source (Arrebola et al., 2011). The toxic moiety of tabtoxin is released by hydrolysis once inside the plant cell, and induces the degradation of chlorophyll, leading to tissue yellowing and chlorosis through the irreversible inhibition of glutamine synthetase (Langston-Unkefer et al., 1987). Phaseolotoxin functions via a similar mechanism, although, in this case, the toxic hydrolytic moiety, octicidine, inhibits ornithine carbamoyl transferase, leading to severe yellowing and tissue chlorosis as a result of metabolic unbalancing of the plant cell (Bender et al., 1999).

Pathogens interfere with hormone physiology in their hosts in order to maximize virulence and overcome plant defences, and manipulation of these internal signalling pathways is clearly central to effective pathogenesis. Many phytotoxic molecules produced by pathogenic microbes have both structural and functional parallels to auxin and other plant hormones. A good example of this is the polyketide plant hormone mimic coronatine, which, in addition to inducing stomatal opening, promotes apoplastic proliferation (Zeng and He, 2010) and enhances disease symptom development. Coronatine is recognized by the plant COI1/JAZ receptor complex and stimulates jasmonic acid (JA) signalling in the plant, in turn suppressing SA-mediated defences (Xin and He, 2013). Many of the biological effects of coronatine closely resemble those induced by jasmonates. These include the stimulation of chlorosis, anthocyanin production, inhibition of root growth and induction of various JA-responsive genes. Coronatine activity is also associated with several non-JA/SA-mediated virulence phenotypes. It contributes to chlorosis and yellowing through stimulation of the plant STAYGREEN gene (Mecey et al., 2011), as well as inducing necrotic cell death by increasing the production of reactive oxygen species (Ishiga et al., 2009).

Several plant pathogens also encode enzyme pathways that directly synthesize plant hormones, providing a direct mechanism for the manipulation of plant hormone signalling. The production of cytokinins, abscisic acid (ABA), indole acetic acid (auxin), JA
and ethylene has been identified in various bacterial species (Robert-Seilaniantz et al., 2011). There is a substantial and complex level of crosstalk between plant hormone signalling pathways, and phytopathogens are able to exploit this by producing or suppressing different hormones, thereby manipulating plant defences and metabolism to their advantage (Robert-Seilaniantz et al., 2011). Operons for the biosynthesis and catabolism of auxin have been found in several phytopathogens, such as Ag. tumefaciens, Pseudomonas savastanoi, P. syringae and Pantocea agglomerans (Spaepen and Vanderleyden, 2011). Increased free auxin levels appear to benefit phytopathogens by suppressing host defence responses, and thus promoting pathogen susceptibility (Navarro et al., 2006; Spaepen and Vanderleyden, 2011). Another plant hormone produced by various phytopathogens is the stress response factor ethylene. Ethylene has been reported to both stimulate and suppress plant defence responses, depending on the particular interactions tested, although, for P. syringae and other virulent phytopathogens, ethylene production generally appears to promote disease (Melotto and Kunkel, 2013).

As the number of sequenced phytopathogen genomes continues to rapidly rise, we are likely to see the increasing importance of comparative genomic analysis tools (Martinez-Garcia et al., 2015), alongside predictive algorithms for small-molecule biosynthetic pathways and enzymes. Perhaps the most well established of these is AntiSMASH 3.0 (Weber et al., 2015), which identifies gene clusters and biosynthetic enzymes for natural products. In silico techniques are already extensively used in natural product operon discovery, and are likely to see increasing use in plant pathogen biology in years to come. Both the existence and, increasingly, the function of plant-pathogenic molecules and peptides will become ever more amenable to in silico analysis, and this method is likely to become the primary approach by which future phytotoxin discoveries are made.

**EFFECTOR PROTEINS AND VIRULENCE**

Successful infection by many phytopathogenic bacteria depends on the secretion of diverse virulence factors, including enzymes, toxins and other host-manipulating molecules, into the extracellular milieu or directly into the host cytosol. Hemibiotrophic bacteria, such as Xanthomonas spp. and P. syringae, use a combination of different secretion systems to enable the export and delivery of effectors, which are secreted proteins that manipulate the structure or function of host components, to the appropriate location for a coordinated and effective infection (Chang et al., 2014). In the next section, we discuss important questions relating to bacterial effectors, their translocation and ongoing attempts to understand the complex molecular interaction between plants and bacterial pathogens (Fig. 4).

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**Fig. 4** Schematic overview of important processes associated with bacterial effectors. (A) The regulation of virulence gene expression by extracellular and intracellular stimuli. (B) Coordinated translocation of effectors by secretion systems, such as the depicted type III secretion system (T3SS), T4SS and T2SS. (C) Effectors target various host processes to promote bacterial proliferation. These processes include immunity, nutrient distribution, hormone signalling, plant metabolism, organelle function and cell structure. (D) Host–pathogen interactions drive evolutionary mechanisms, leading to the expansion and diversification of virulence gene families and shaping the effector repertoire of pathogen populations.

**REGULATION, DYNAMICS AND HIERARCHY OF EFFECTOR TRANSLOCATION**

Coordination between available secretion systems contributes markedly to phytopathogen versatility. However, we currently have only a limited knowledge of how the different secretion systems in a bacterial cell are regulated in response to changes in external stimuli (Fig. 4A), with a few exceptions. In P. aeruginosa, the post-transcriptional regulator RsmA acts as a molecular switch between acute and chronic infections by negatively affecting the translation of pel, psl and T6SS mRNAs and by promoting flagellar, T4SS and T3SS gene transcription (Brenic and Lory, 2009; Moscoso et al., 2011). Similarly, the sensor kinases RetS and LadS antagonistically regulate T6SS and T3SS in Pss B728a (Records and Gross, 2010). QS plays a critical role in the regulation of T2SS in Xanthomonas (Jha et al., 2005). Regulation of the so-called hrp (hypersensitive response and pathogenicity) regulon, which includes T3SS structural genes, T3SS-associated regulators and many T3Es, has also been studied extensively (Büttner and Bonas, 2010; Genin and Denny, 2012; Tampakaki et al., 2010).

In addition to the transcriptional regulation of T3SS, pathogens ensure hierarchical and temporal control of T3E delivery during infection by post-translational mechanisms (Galán et al., 2014; Lohou et al., 2013). Salmonella enterica uses a cytoplasmic sorting platform involving chaperones to recruit and secrete T3Es in a well-defined order (Lara-Tejero et al., 2011). Bacterial plant pathogens also employ hpa-associated (hpa) proteins, such as chaperones and export control proteins, for coordinated delivery of T3Es into host cells (Lohou et al., 2013). Recently, a proteomics approach to the R. solanacearum secreteme identified differential
T3E secretion patterns in *hpaB*, *hpaD* and *hpaG* mutant strains, which affected bacterial virulence and supports the idea of the hierarchical delivery of T3Es (Lonjon *et al*., 2016). Mechanistically, it has been suggested that the global T3E export chaperone HpaB interacts with HpaA to select and guide T3Es to the T3SS-associated ATPase HrcN for translocation (Büttnier *et al*., 2006; Chang *et al*., 2014). Interestingly, the secondary messenger cdG has recently been shown to allosterically regulate HrcN activity, suggesting a role for this signalling molecule in the control of T3E translocation dynamics (Trampari *et al*., 2015). Coordinated secretion of T3Es is important for *Pss* B728a during epiphytic growth as, in this phase, HopAA1 and HopZ3 seem to play a special role in the colonization of distinctive leaf surfaces (Lee *et al*., 2012). To gain a detailed insight into the temporal dynamics of T3E secretion, a microscopy-based assay tracking translocation of individual T3Es has been developed for *Escherichia coli* (Mills *et al*., 2013). This technique could be readily adapted for plant pathogens, and could potentially shed further light on exactly how these bacteria deploy their T3Es during the infection process.

**DIFFERENT MODES OF EFFECTOR TRANSLLOCATION**

Coordinated translocation of proteins and other cargo molecules requires complex transport systems (Fig. 4B). There are at least six secretion systems employed by Gram-negative bacteria to deliver proteins into the extracellular environment or directly into a recipient cell. These secretion pathways, type I to type VI, differ significantly in their composition, recognition of cargo and function (Büttnier and Bonas, 2010; Chang *et al*., 2014). T3SS, in particular, plays an essential role in pathogenicity, as mutants in many Gram-negative phytopathogenic species fail to cause disease in susceptible plants. Given the importance of T3SS during pathogenesis, specific attention has been dedicated to identify T3E repertoires and their biological function. However, other secretion systems have been shown to be relevant and, in some cases, crucial for pathogenesis. Often, multiple secretion systems work alongside each another to achieve full virulence (Preston *et al*., 2005). Future research will reveal the effector repertoires and *in planta* regulation of the complete phytopathogenic secretome, contributing to a better understanding of bacterial pathogenesis.

The advent of whole-genome sequencing has made it possible to identify an organism’s complete T3E repertoire by making use of conserved elements and characteristic signatures in the genome (Lindeberg *et al*., 2006). Over recent years, various genome mining approaches, coupled with functional characterization, have allowed us to build up a provisional T3E super-repertoire for *P. syringae* (O’Brien *et al*., 2011), *Xanthomonas* spp. (White *et al*., 2009) and *R. solanacearum* (Peeters *et al*., 2013; Roux *et al*., 2015). Although the majority of T3E families from the *P. syringae* species complex have been described (Baltrus *et al*., 2011), new T3E families are continuously being found (Hockett *et al*., 2014; Matas *et al*., 2013), with low-cost draft genomes enabled by next-generation sequencing likely to permit more extensive sampling from different crop species, alternative hosts or environmental samples in the future. Community efforts have established a unified nomenclature, built up databases of known T3E families and generated bioinformatics tools for extensive genomic analysis (Lindeberg *et al*., 2005; Peeters *et al*., 2013), which should greatly assist future efforts to identify and characterize effector proteins.

Pathogenic and non-pathogenic bacteria use T2SS to deliver proteins into the extracellular environment (Johnson *et al*., 2006). A large number of T2ES are major virulence factors, such as toxins, proteases and cell wall-degrading enzymes, contributing to virulence in animals and plants. Plant pathogens containing a T2SS include numerous members of the genera *Erwinia*, *Pseudomonas*, *Ralstonia* and *Xanthomonas* (Cianciotto, 2005; Jha *et al*., 2005). All sequenced *Xanthomonas* species and many other Gram-negative bacteria contain the lipA gene encoding a type II secreted lipase, whose function has been shown to be required for the full virulence of *X. oryzae* pv. *oryzae* (Aparna *et al*., 2009) and *X. campestris* pv. *vesicatoria* (Tamir-Ariel *et al*., 2012).

T4SSs have been found in diverse bacterial genera, and translocate DNA and proteins into the extracellular environment or directly into recipient cells (Bhatti *et al*., 2013). T4SS cargo can include proteins, DNA–protein complexes and fully assembled protein macromolecular complexes (Bhatti *et al*., 2013). Among plant pathogens, T4SS is mostly known in *Ag. tumefaciens* for translocation of T-DNA into host cells to enable tumour induction (Gohike and Deeken, 2014). Recently, it has been shown that *X. citri* uses a T4SS for toxin translocation to kill other bacteria, providing a growth advantage in mixed communities (Souza *et al*., 2015). Genome analysis has also identified putative T1SS, T5SS and T6SS in diverse plant pathogens; however, their biological roles are currently poorly understood, with no strong virulence phenotype associated with any of these secretion systems so far (Chang *et al*., 2014; Preston *et al*., 2005; Records and Gross, 2010). Future genome comparison analyses and refined bioinformatics approaches will tell us more about the variation within the different phytopathogen secretory pathways and their associated effectors, and will hopefully reveal their roles during pathogenesis, and also during environmental persistence (Bhatti *et al*., 2013; Korotkov *et al*., 2012; Sarris *et al*., 2010). Research on non-culturative phytopathogens, such as the obligate intracellular *Phytoplasma*, have particularly benefited from genome mining approaches. This has enabled the discovery of many secreted effectors with crucial functions for the pathogen lifecycle (MacLean *et al*., 2011; Sugio and Hogenhout, 2012).

**FUNCTION OF T3ES**

Effectors interfere with plant processes to benefit microbial fitness in the host environment. Genome analysis has revealed...
whole suites of effector proteins, whereas functional characterization has revealed that a major role of T3Es is the suppression of plant immune responses (Block and Alfano, 2011; Cunnac et al., 2009; Macho and Zipfel, 2015). The molecular interplay between bacterial T3Es and plant immune components has been summarized in a model describing the evolutionary arms race of pathogens and plants (Dodds and Rathjen, 2010; Jones and Dangl, 2006). Although T3Es suppressing plant immunity have been studied in great detail, other host processes that are frequently targeted by effectors during the course of an infection, but are not directly linked to immunity, have been less well characterized. These processes include nutrient distribution, metabolism, organelle function and cell development (Fig. 4C) (Macho, 2016). In addition to classifying effectors by their degree of conservation, assigning them to effector-targeted pathways (ETPs) according to their function might provide better insights into the complex processes and determinants underlying virulence, as well as host and tissue specificity (Win et al., 2012).

The synchronization of host physiological status and bacterial metabolic pathways is necessary for optimal pathogen growth. To this end, transcription activator-like (TAL) effectors are encoded by members of Xanthomonas andRalstonia spp., and function as plant transcription factors to promote virulence and to establish a compatible environment to support bacterial growth (Bogdanove et al., 2010). For example, the rice pathogen X. oryzae pv. oryzae manipulates the expression genes encoding for SWEET sugar transporters by multiple TAL effectors (Chen et al., 2010; Streubel et al., 2013). Interestingly, Pto DC3000 also induces T3SS-dependent expression of the SWEET genes during infection of Arabidopsis thaliana (Chen et al., 2010). The induced sugar influx to plant cells to the apoplast might also result in water release to maintain osmotic homeostasis in the tissue, further changing the environment in the intercellular space and specifically benefiting bacterial infection (Macho, 2016). Pathogens can also modify plant tissue by manipulating developmental regulators or plant hormone signalling. For example, the TAL effector AvrBs3 from X. campestris induces expression of the plant cell size regulator UPA20, resulting in enlarged mesophyll cells, which may promote bacterial growth and dissemination (Kay et al., 2007). Various T3Es from P. syringae, such as AvrRpt2, HopX1 and AvrPtoB, affect the auxin, JA and ABA signalling pathways, respectively (Cui et al., 2013; De Torres Zabala et al., 2009; Gimenez-Ibanez et al., 2014). Interference with hormone signalling by deployment of T3Es is widespread among phytopathogens and may directly or indirectly disrupt immune responses, but also impacts plant physiology and metabolism, thereby creating a niche for bacterial propagation (Kazan and Lyons, 2014). Additional ETPs are the plant cytoskeleton or cellular trafficking pathways, with effector deployment leading to altered cell structure and function. These phenotypes have been associated with suppression of defence responses (Kang et al., 2014; Nomura et al., 2011); however, the remodelling of cell integrity and transport pathways can also have broader implications for plant homeostasis. T3Es have also been shown to directly affect plant metabolism by interfering with biosynthetic pathways for secondary metabolites. For example, Pantoea stewartii secretes a T3E of the AvrE family, which perturbs phenylpropanoid metabolism and promotes pathogen virulence (Asselin et al., 2015). Chloroplasts and mitochondria are involved in many aspects of plant physiology and are consequently an obvious target for T3Es. Among others, HopK1 from P. syringae localizes to the chloroplast, suppresses photosynthesis and contributes to virulence (Li et al., 2014). Similarly, P. syringae HopG1 perturbs mitochondrial function and plant development and probably contributes to bacterial virulence (Block et al., 2010). These examples highlight the various ways in which pathogens use T3Es to manipulate their hosts and to generate a suitable environment for survival, growth and dispersal. Finally, the manipulation of plant development represents an effective virulence strategy. Agrobacterium tumefaciens uses a T4SS to transfer DNA (T-DNA) together with virulence proteins into plant cells. The T-DNA integrates into the genome and governs host cell reprogramming, leading to altered metabolism and morphology (Gohlke and Deeken, 2014).

Typically, the T3E repertoire is functionally redundant and deletion of individual T3Es often has no effect on virulence. Systematic mutagenesis, combined with virulence phenotyping, has been used to dissect the function and importance of individual T3Es in Pto DC3000, leading to the identification of a minimal T3E repertoire, which is sufficient for wild-type-level infection of Nicotiana benthamiana (Cunnac et al., 2011; Kvitko et al., 2009). A major future challenge will be to elucidate what is required in a functional T3E repertoire to enable pathogenicity (Lindeberg et al., 2012). To address this, genomics and computational analysis must be coupled to functional and mechanism studies to characterize the role of T3Es in the plant cell. Improved biochemical, proteomics and cell biology techniques, together with high-throughput methods, such as transient assays in heterologous systems to screen for disease symptoms (Almeida et al., 2009; Cunnac et al., 2011), or to determine subcellular localization and host interactors (Petre et al., 2015), will also help us to understand the function and evolution of T3E repertoires.

**EVOLUTION OF EFFECTOR REPERTOIRES**

Phytopathogens, such as P. syringae or the Xanthomonas genus, have a broad host range, but pathogen variants (pathovars) or single species are restricted to one or a few plants, reflecting strong host adaptation (Lindeberg et al., 2009; Ryan et al., 2011). Understanding the factors that influence the host ranges of pathogenic
bacteria and the underlying mechanism of host range evolution is a major challenge (Lindeberg et al., 2012).

Genome comparison between phylogenetically related strains provides insights into the evolutionary processes shaping the T3E repertoire, and its contribution to host and/or tissue specificity for individual strains. In this approach, conserved T3Es across phylogenetically diverse strains represent core T3Es that provide basal virulence functions in all hosts, whereas variable T3Es are important for individual host specificity (Lindeberg et al., 2012). A comparison between 19 P. syringae strains representing the three major subclades revealed a core set of the four bona fide T3Es: HopM, AvrE, HopAA and HopI (Baltrus et al., 2011). However, the authors could not pinpoint factors determining host specialization because of the high diversity of virulence components and the variability of T3E repertoires among different pathovars (Baltrus et al., 2011).

The molecular arms race between pathogens and hosts drives the evolution of T3Es as immune suppressors (Guo et al., 2009; Lindeberg et al., 2012; Win et al., 2012). However, the evolutionary purpose of the high degree of functional redundancy observed in T3E repertoires is not fully understood. Targeting a plant defence pathway multiple times might ensure the effective disruption of plant defences over the course of an infection and would provide a robust long-term virulence strategy in case a specific T3E is recognized by a host species and turned into an avirulence effector. A model for two-stage co-evolution has been suggested, in which variable T3Es evolve to protect the function of core T3Es, leading to highly redundant repertoires (Lindeberg et al., 2012). Furthermore, a pathogen population benefits from heterogeneous, strain-specific T3E repertoires when disease-resistant host plants emerge. Under these conditions, individual strains with virulent T3Es for the new host are positively selected and help to ensure the survival of the pathogen population. This evolutionary ‘bet hedging’ strategy would allow a population to include redundant T3Es to increase flexibility in response to evolving host plants (Win et al., 2012).

No clear pattern was detected in the T3E repertoire that could be associated with the specificity of P. syringae pathovars (Lindeberg et al., 2012), R. solanacearum strains (Genin and Denny, 2012) or Xanthomonas spp. (Roux et al., 2015), underlining the complex genetic basis for adaptation to specific hosts. This is also exemplified by the diverse T3E repertoires from individual isolates. The closely related Pto strains DC3000 and T1 can both infect tomato plants with a shared T3E repertoire of 14 T3E genes, in contrast with 15 and 11 additional strain-specific T3E genes, respectively (Almeida et al., 2009). To address the genetic basis of why Pto DC3000, but not T1, is able to infect A. thaliana, mutagenesis and functional T3E characterization were performed, but could not fully explain the compatibility of Pto DC3000 with A. thaliana. Additional factors might be involved in pathogenesis on A. thaliana (Almeida et al., 2009; Sohn et al., 2012). Interestingly, P. syringae phylogroup II strains contain the smallest number of T3Es, but encode operons for many phytotoxins compared with the other groups (Baltrus et al., 2011).

Diversification of the T3E repertoire is an important long-term virulence strategy, and pathogenic bacteria have evolved several mechanisms for the acquisition and loss of T3E genes (Fig. 4D) (Jackson et al., 2011); therefore, an analysis based on only the presence or absence of T3E genes might not provide the full picture. Several examples have been reported in which pathogenic bacteria modify T3E gene expression, protein activity, target specificity or detection by R proteins (Ma and Guttman, 2008). Comparative genomic analysis complements research into molecular plant–microbe interactions, as it can provide additional information about discrete residues in a given T3E under diversifying selection imposed by the host plant (Baltrus et al., 2011).

A robust association between host range evolution and T3E repertoire requires many genomic sequences and careful sampling. A population genomics approach, in which isolates ideally represent a defined population, helps us to understand how host shifts and specialization occur and what evolutionary paths are available when a pathogen adapts to a specific host (Vinatzer et al., 2014). Ultimately, examination of the evolution of T3Es provides a very detailed picture of the adaptation process, as T3Es are highly variable and evolve rapidly because of strong selection pressure imposed by plant immunity components (Ma and Guttman, 2008). Progress in bioinformatics methods for T3E detection, together with functional information on T3Es and population genomics analysis determining phylogenetic relationships between isolates, will enable us to monitor pathogen populations in the field and to predict epidemic outbreaks in the future (Vinatzer et al., 2014).

CONCLUSION

Although the future challenges faced by plant pathology researchers are certainly daunting, new research tools, including techniques facilitated by the rise of next-generation sequencing and advanced computational analysis, have already enabled us to investigate plant pathogenesis at a resolution that would have been impossible just a few years ago. Future research is likely to continue to move towards a global understanding of the pathogen–plant interaction, and promises to keep us busy for many years to come.

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REFERENCES

Abel, S., Abel Zur Wiesch, P., Chang, H.H., Davis, B.M., Lipstitch, M. and Waldor, M.K. (2013) Sequence tag-based analysis of microbial population dynamics. Nat. Methods, 12, 223–226.

Allesin-Holtm, M., Biebl, K.B., Yang, L., Klausen, M., Webb, J.S., Kjelleberg, S., Molin, S., Givskov, M. and Tolker-Nielsen, T. (2006) A characterization of DNA release in Pseudomonas aeruginosa cultures and biofilms. Mol. Microbiol. 59, 1114–1128.

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., Angott, A., Martin, G.B., Jones, J.D., Collmer, A., Setubal, J.C. and Wild, C. (2006) A characterization of a type III effector 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.

An, S.Q., Allan, J.H., McCarthy, Y., Febrer, M., Dow, J.M. and Ryan, R.P. (2014b) Novel cyclic di-GMP effectors of the YjaQ protein family control bacterial virulence. PLoS Pathog. 10, e1004429.

Andrews, J.H. and Harris, R.F. (2000) The ecology and biogeography of microorganisms on plant surfaces. Annu. Rev. Phytopathol. 38, 145–180.

Apana, G., Chatterjee, A., Sonit, R.V. and Sanakanarayan, R. (2009) A cell wall-degrading extracellular of Xanthomonas oryzae requires a unique substate recognition module for pathogenesis on rice. Plant Cell 21, 1860–1873.

Arrebola, E., Cazorla, F.M., Perez-Garcia, A. and de Vicente, A. (2015) Perturbation of maize phenylpropanoid metabolism by an AvrE effectors: virulence targets or guarded decoys? Mol. Plant 8, 1298–1313.

Barch, A.Y., Shimada, B.K., Mullin, S.W., Dunlap, C.A., Bowman, M.J. and Lindow, S.E. (2012) Pseudomonas syringae coordinates production of a motility-enabling surfactant with flagellar assembly. J. Bacteriol. 194, 1287–1298.

Böttner, D. and Bonas, U. (2010) Regulation and secretion of Xanthomonas virulence factors. FEMS Microbiol. Rev. 34, 107–133.

Böttner, D., Lorenz, C., Weber, E. and Bonas, U. (2006) Targeting of two effector protein classes to the type III secretion system by a HpaC- and HpaB-dependent protein complex from Xanthomonas campestris pv. vesicatoria. Mol. Microbiol. 59, 513–527.

Chang, J.H., Desveaux, D. and Creason, A.L. (2014) The ABCs and 123s of bacterial secretion systems in plant pathogenesis. Annu. Rev. Phytopathol. 52, 317–345.

Chang, W.S., van de Mortel, M., Nielsen, L., de Guzman, G.N., Li, X.H. and Halverson, L.J. (2007) Alginate production by Pseudomonas putida creates a hydrated microenvironment and contributes to biofilm architecture and stress tolerance under water-limiting conditions. J. Bacteriol. 189, 8290–8299.

Chatterjee, S., Almeida, R.P. and Lindow, S. (2008a) Living in two worlds: the plant and insect lifestyles of Xylella fastidiosa. Annu. Rev. Phytopathol. 46, 243–271.

Chatterjee, S., Newman, K.L. and Lindow, S.E. (2008b) Cell-to-cell signaling in Xylella fastidiosa suppresses movement and xylem vessel colonization in grape. Mol. Plant–Microbe Interact. 21, 1309–1315.

Chatterjee, S., Wistrom, C. and Lindow, S.E. (2008a) A cell–cell signaling sensor is required for virulence and insect transmission of Xylella fastidiosa. Proc. Natl. Acad. Sci. USA 105, 2670–2675.

Chen, L.Q., Hou, B.H., Lalonde, S., Takahana, H., Hartung, M.L., Xu, Q.Q., Guo, W.J., Kim, J.G., Underwood, W., Chaudhuri, B., Cermak, D., Antony, G., White, F.F., Somerville, S.C., Mudgett, M.B. and Frommer, W.B. (2010) Sugar transporters for intercellular exchange and nutrition of pathogens. Nature, 468, 527–532.

Chen, K.H., Lee, Y.C., Tu, Z.L., Chen, C.H., Tseng, Y.H., Yang, J.M., Ryan, R.P., McCarthy, Y., Dow, J.M., Wang, A.H. and Chou, S.H. (2010) The cAMP receptor-like protein CLP is a novel c-di-GMP receptor linking cell–cell signaling to virulence gene expression in Xanthomonas campestris. J. Mol. Biol. 396, 646–662.

Cianciotto, N.P. (2005) Type II secretion: a protein secretion system for all seasons. Trends Microbiol. 13, 581–588.

Cui, Y., Wu, S., Sun, W., Coaker, G., Kunkel, B., He, P. and Shan, L. (2013) The Pseudomonas syringae type III effector AvrRpt2 promotes pathogen virulence via stimulating Arabidopsis auxin/indole acetic acid protein turnover. Plant Physiol. 162, 1018–1029.

Cunnack, 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.

Cunnack, S., Chakravarthy, S., Kvitko, B.H., Russell, A.B., Martin, G.B. and Collmer, A. (2011) Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in Pseudomonas syringae. Proc. Natl. Acad. Sci. USA 108, 2975–2980.

De Torres Zabala, M., Bennett, M.H., Truman, W.H. and Grant, M.R. (2013) Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in Pseudomonas syringae. Proc. Natl. Acad. Sci. USA 108, 2975–2980.

Delmotte, N., Knief, C., Chaffron, S., Innerleben, G., Roschitzki, B., Schlapbach, R., von Mering, C. and Vorholt, J.A. (2009) Community proteogenomics reveals insights into the physiology of phytophthora bacteria. Proc. Natl. Acad. Sci. USA 106, 16 428–16 433.

Dovnjak, S., Urbach, J.M., Drenkard, E., Bush, J., Feinbaum, R., Ausubel, J.L., Traficante, D., Risch, M., Kocks, C., Fischbach, M.A., Priebe, G.P. and Ausubel, F.M. (2013) Trehalose biosynthesis promotes Pseudomonas aeruginosa pathogenicity in plants. PLoS Pathog. 9, e1003217.
Dodd, P.N. and Rathjen, J.P. (2010) Plant immunity: towards an integrated view of plant–pathogen interactions. Nat. Rev. Genet. 11, 539–548.

Dunger, G., Relling, V.M., Tondo, M.L., Barreras, M., Ielpi, L., Orellano, E.G. and Ottado, J. (2007) Xanthan is not essential for pathogenicity in citrus canker but contributes to Xanthomonas epiphytic survival. Arch. Microbiol. 188, 127–135.

Engl, C., Waite, C.J., McKenna, J.F., Bennett, M.H., Hamann, T. and Buck, M. (2014) ChpB, a diacylglycarnyl cyclase from Pseudomonas syringae pv. tomato DC3000, suppresses the pathogen-associated molecular pattern flagellin, increases extracellular polysaccharides, and promotes plant immune evasion. MBio, 5, e01114–e01168.

Freeman, B.C., Chen, C. and Beattie, G.A. (2014) Identification of the trehalose biosynthetic loci of Pseudomonas syringae and their contribution to fitness in the phyllosphere. Environ. Microbiol. 12, 1486–1497.

Freeman, B.C., Chen, C., Xu, Y., Nielsen, L., Peterson, K. and Beattie, G.A. (2013) Physiological and transcriptional responses to osmotic stress of two Pseudomonas syringae strains that differ in epiphytic fitness and osmotolerance. J. Bacteriol. 195, 4742–4752.

Gaignard, J.L. and Luisetti, J. (1993) Pseudomonas-syringae, an epithelial cell nucleation active and phytopathogenic bacterium. Agronomie, 13, 333–370.

Gal, M., Preston, G.M., Massey, R.C., Spier, A.J. and Rainey, P.B. (2003) Genes encoding a cellulosic polymer contribute toward the ecological success of Pseudomonas fluorescens SBW25 on plant surfaces. Mol. Ecol. 12, 3109–3121.

Galán, J.E., Lara-Tejero, M., Marlovits, T.C. and Wagner, S. (2014) Bacterial type III secretion systems: specialized nanomachines for protein delivery into target cells. Annu. Rev. Microbiol. 68, 415–438.

Gamborski, C.P., Campbell, R.L., Walker, V.K. and Davies, P.L. (2011) Novel dimeric beta-helical model of an ice nucleation protein with bridged active sites. BMC Struct. Biol. 11, 36.

Genin, S. and Denny, T.P. (2012) Pathogenomics of the Raistonia solanacearum species complex. Annu. Rev. Phytopathol. 50, 67–89.

Gods, S., Sims, I.M., Morraldis, M.F. and Rehm, B.H. (2015) Bacterial compounds controlling growth of the plant pathogen Pseudomonas syringae pv. actinidiae, which forms a biosynthetic compound of a novel exopolysaccharide. Appl. Environ. Microbiol. 81, 4026–4036.

Giménez-Ibáñez, S., Boter, M., Fernández-Barbero, G., Chini, A., Rathjen, J.P. and Solano, R. (2014) The bacterial effector HopX1 targets TA2 transcriptional repressors to activate jasmonate signaling and promote infection in Arabidopsis. PLoS Biol. 12, e1001792.

Gohlike, J. and Deeken, R. (2014) Plant responses to Agrobacterium tumefaciens and crown gall development. Front. Plant Sci. 5, Article 155.

Gudesblat, G.E., Torres, P.S. and Vojnov, A.A. (2009a) Stomata and pathogens: warfare at the gates. Plant Signal. Behav. 4, 1114–1116.

Gudesblat, G.E., Torres, P.S. and Vojnov, A.A. (2009b) Xanthomonas campestris overcomes Arabidopsis stomatal innate immunity through a DSF cell-to-cell signal-regulated virulence factor. Plant Physiol. 149, 1017–1027.

Guo, M., Tian, F., Wamboldt, Y. and Alfano, J.R. (2009) The majority of the type III effector inventory of Pseudomonas syringae pv. tomato DC3000 type III secretion effector gene set reveals functional overlap among effectors. PLoS Pathog. 5, e1000388.

Langston-Unkefer, P.J., Robinson, A.C., Knight, G.R. and Durbin, R.D. (1987) Inactivation of pea seed glutamine synthetase by the toxin, tabtoxinine-beta-lactam. J. Biol. Chem. 262, 1608–1613.

Lara-Tejero, M., Kato, J., Wagner, S., Liu, X. and Galán, J.E. (2011) A sorting platform determines the order of protein secretion in bacterial type III systems. Science, 331, 1188–1191.

Laue, H., Schenk, A., Li, H., Lambersen, L., Neu, T.R., Molin, S. and Ullrich, M.S. (2006) Contribution of alginate and levan production to biofilm formation by Pseudomonas syringae. Microbiology, 152, 2909–2918.

Leduc, J.L. and Roberts, G.P. (2009) Cyclic di-GMP allosterically inhibits the CRP-like protein (Cip) of Xanthomonas axonopodis pv. citri. J. Bacteriol. 191, 7121–7122.

Lee, D.H., Kim, J.B., Lim, J.A., Han, S.W. and Heu, S. (2016) The evolution of Xanthomonas campestris pv. tomato carotovorum subsp. brasiliensis isolated in Korea. Plant Pathol. J. 30, 117–124.

Lee, J., Teitel, G.M., Munkvold, K., del Pozo, O., Martin, G.B., Michelmore, R.W. and Greenberg, J.T. (2012) Type II secretion and effectors shape the survival and growth pattern of Pseudomonas syringae on leaf surfaces. Plant Physiol. 158, 1803–1818.

Lee, Y.W., Jin, S., Sim, W.S. and Nester, E.W. (1995) Genetic evidence for direct sensing of phenolic compounds by the VirA protein of Agrobacterium tumefaciens. Proc. Natl. Acad. Sci. USA, 92, 12435–12440.

Li, G., Froehlich, J.E., Elowsky, C., Misanne, J., Ostos, A.C., Zhang, C., Awada, T. and Alfano, J.R. (2014) Distinct Pseudomonas type-III effectors use a cleavable transit peptide to target chloroplasts. Plant J. 77, 310–321.

Lindeberg, M., Stavrínides, J., Chang, J.H., Alfano, J.R., Collmer, A., Dani, J.L., Greenberg, J.T., Mansfield, J.W. and Guttmann, D.S. (2005) Proposed guidelines for a unified nomenclature and phylogenetic analysis of type III hop effector proteins in the plant pathogen Pseudomonas syringae. Mol. Plant–Microbe Interact. 18, 275–282.

Lindeberg, M., Cattinhou, S., Myers, C.R., Schechter, L.M., Schneider, D.J. and Collmer, A. (2006) Closing the circle on the discovery of genes encoding Hrp region members and type III secretion system effectors in the genomes of three model Pseudomonas syringae strains. Mol. Plant–Microbe Interact. 19, 1151–1158.

Lindervallberg, M., Cunnac, S. and Collmer, A. (2009) The evolution of Pseudomonas syringae host specificity and type III effector repertoires. Mol. Plant Pathol. 10, 767–775.
Lindeberg, M., Cunnac, S. and Collamer, A. (2012) Pseudomonas syringae type III effector repertoires: last words in endless arguments. Trends Microbiol. 20, 199–208.

Little, R.H., Grela, L., Saalbach, G., Howat, A.M., Pfeilmeier, S., Trampari, E. and Malone, J.G. (2010) Adaptive remodeling of the bacterial proteome by specific ribosomal modification regulates Pseudomonas infection and niche colonization. PLoS Genet. 12, e1005837.

Lohou, D., Lonjon, F., Genin, S. and Vailleau, F. (2013) Type III chaperones & Co in bacterial plant pathogens: a set of specialized bodyguards mediating effector delivery. Front. Plant Sci. 4, 435.

Lonjon, F., Turner, M., Henry, C., Rengel, D., Lohou, D., van de Rijn, Q., de Vicente, A., Matas, I.M., Castaño-Ojeda, M.P., Aragón, I.M., Antúnez-Lamas, M., Murillo, J., Rodríguez-Palenzuela, P., López-Solanilla, E. and Ramos, C. (2013) Translocation and functional analysis of Pseudomonas savastanoi pv. savastanoi NCPPB 3335 type III secretion system effector reveals two novel effector families of the Pseudomonas syringae syringae complex. Mol. Plant–Microbe Interact. 27, 424–436.

McDow, Y., Doy, J.M. and Ryan, R.P. (2011) The Ax21 protein is a cell–cell signal that regulates virulence in the nosocomial pathogen Stenotrophomonas maltophilia. J. Bacteriol. 193, 6375–6378.

Meece, C., Hauck, P., Trapp, M., Pumpin, A., Plovanich, A., Yao, J. and He, S.Y. (2011) A critical role of STAYGREEN/Mendel’s I locus in controlling disease symptom development during Pseudomonas syringae pv. tomato infection of Arabidopsis. Plant Physiol. 157, 1965–1974.

Melotto, M. and Kunkel, B.N. (2013) Virulence strategies of plant pathogenic bacteria. In: The Prokaryotes – Prokaryotic Physiology and Biochemistry (Rosenberg, E., ed.), pp. 61–75. Heidelberg: Springer-Verlag.

Melotto, M., Underwood, W., Koczan, J., Nomura, K. and He, S.Y. (2006) Plant stomata function in innate immunity against bacterial invasion. Cell, 126, 969–980.

Mills, E., Baruch, K., Aviv, G., Nitzan, M. and Rosenshine, I. (2013) Dynamics of the type III secretion system activity of enteropathogenic Escherichia coli. mBio, 4, e00303.

Molina, L.G., da Fonseca, G.C., de Morais, G.L., de Oliveira, L.F., de Carvalho, J.B., Kulcheski, F.R. and Margis, R. (2012) Metatranscriptomic analysis of small RNAs present in soybean deep sequencing libraries. Genet. Mol. Biol. 35, 292–303.

Moscoso, J.A., Mikkelsen, H., Heeb, S., Williams, P. and Filloux, A. (2011) The Pseudomonas aeruginosa sensor Ret switches type III and type VI secretion via c-di-GMP signaling. Environ. Microbiol. 13, 3128–3138.

Moscoso, J.A., Jaeger, T., Valentini, M., Hui, K., Jenal, U. and Filloux, A. (2014) The diguanylate cyclase SadC is a central player in Gac/Sm-mediated biofilm formation in Pseudomonas aeruginosa. J. Bacteriol. 196, 4081–4088.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O. and Jones, J.D. (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science, 312, 436–439.

Newman, K.L., Almeida, R.P., Purcell, A.H. and Lindow, S.E. (2004) Cell–cell signaling controls Xylella fastidiosa interactions with both insects and plants. Proc. Natl. Acad. Sci. USA, 101, 1737–1742.

Nguyen, L.C., Taguchi, F., Tran, Q.M., Naito, K., Yamamoto, M., Ohnishi-Kameyama, M., Ono, H., Yoshida, M., Chiku, K., Ishii, T., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinoise, Y. (2012) Type IV pilin is glycosylated in Pseudomonas syringae pv. tabaci 6005 and is required for surface motility and virulence. Mol. Plant Pathol. 13, 764–774.

Nomura, K., Meccey, C., Lee, Y.N., Imboden, L.A., Chang, J.H. and He, S.Y. (2011) Effector-triggered immunity blocks pathogen degradation of an immunity-associated vesicle traffic regulator in Arabidopsis. Proc. Natl. Acad. Sci. USA, 108, 10774–10779.

O’Brien, H.E., Thakur, S. and Guttman, D.S. (2011) Evolution of plant pathogen–host interactions in Pseudomonas syringae: a genomics perspective. Annu. Rev. Phytopathol. 49, 269–289.

Pappas, K.M. and Winans, S.C. (2003) A LuxR-type regulator from Agrobacterium tumefaciens elevates Ti plasmid copy number by activating transcription of plasmid replication genes. Mol. Microbiol. 48, 1059–1073.

Peeters, N., Carrère, S., Anisimova, M., Plener, L., Cazalé, A.C. and Genin, S. (2013) Repertoire, unified nomenclature and evolution of the Type III effector gene family in the Ralstonia solanacearum species complex. BMC Genomics, 14, 1–19.

Pel, M.J., van Dijken, A.J., Bardoeil, B.W., Seidl, M.F., van der Ent, S., van Strijp, J.A. and Pieterse, C.M. (2014) Pseudomonas syringae evades host immunity by degrading flagellin monomers with alkaline protease AprA. Mol. Plant–Microbe Interact. 27, 603–610.

Peng, W.T., Lee, Y.W. and Nester, E.W. (1998) The phenolic recognition profiles of the Agrobacterium tumefaciens VirA protein are broadened by a high level of the sugar binding protein ChvE. J. Bacteriol. 180, 5632–5638.

Perez-Mendoza, D., Aragon, I.M., Prada-Ramirez, H.A., Romero-Jimenez, L., Ramos, C., Gallegos, M.T. and Sanjuan, J. (2014) Responses to elevated c-di-GMP levels in mutualistic and pathogenic plant-interacting bacteria. PLoS One, 9, e91645.

Pisci, E.C., Milbank, J.B., Pearson, J.P., McKnight, S., Kende, A.S., Greenberg, E.P. and Iglewski, B.H. (1998) Quorum sensing in the cell–to-cell communication system of Pseudomonas aeruginosa. Proc. Natl. Acad. Sci. USA, 96, 11229–11234.

Petre, B., Saunders, D.G.O., Sklenar, J., Lorrain, C., Win, J., Duplessis, S. and Kamoun, S. (2015) Candidate effector proteins of the rust pathogen Melampsora lori-populina target diverse plant cell compartments. Mol. Plant–Microbe Interact. 28, 689–700.

Pfeilmeier, S., Saur, I.M., Ratihen, J.P., Zipfel, C. and Malone, J.G. (2016) High levels of cyclic-di-GMP in plant-associated Pseudomonas correlate with evasion of plant immunity. Mol. Plant–Microbe Interact. 27, 521–531.

Phan Tran, D., Herbst, F. and Montag, F. (2011) Xanthomonas. Available at https://vimeo.com/34378870. Vimeo.

Pierce, B.K., Voegel, T. and Kirkpatrick, B.C. (2014) The Xylella fastidiosa PDI063 protein is secreted in association with outer membrane vesicles. PLoS One, 9, e113504.

Prada-Ramirez, H.A., Perez-Mendoza, D., Felipe, A., Martinez-Granero, F., Rivilla, R., Sanjuan, J. and Gallegos, M.T. (2014) AnmZ regulates cellulose production in Pseudomonas syringae pv. tomato DC3000. Mol. Microbiol. 99, 960–977.

Preston, G.M., Studholme, D.J. and Caleferrari, I. (2005) Profiling the secretomes of plant pathogenic Proteobacteria. FEMS Microbiol. Rev. 29, 331–360.

Qian, G., Zhou, Y., Zhao, Y., Song, Z., Wang, S., Fan, J., Hu, B., Venturi, V. and Liu, F. (2013) Proteomic analysis reveals novel extracellular virulence-associated proteins and functions regulated by the diffusible signal factor (DSF) in Xanthomonas oryzae pv. oryzicola. J. Proteome Res. 12, 3327–3341.

Quinones, B., Pujol, C.J. and Lindow, S.E. (2004) Regulation of AHL production and its contribution to epiphytic fitness in Pseudomonas syringae. Mol. Plant–Microbe Interact. 17, 521–531.

Quinones, B., Dulla, G. and Lindow, S.E. (2005) Quorum sensing regulates exopolsaccharide production, motility, and virulence in Pseudomonas syringae. Mol. Plant–Microbe Interact. 18, 682–693.

Records, A.R. and Gross, D.C. (2010) Sensor kinases Rpf and Lrp regulate Pseudomonas syringae type VI secretion and virulence factors. J. Bacteriol. 192, 3584–3596.

Remzi, M., Copini, P., Toddei, A.B., Rossetti, A., Gallipoli, L., Mazzaglia, A. and Balestra, G.M. (2012) Bacterial canker on kiwifruit in Italy: anatomical changes in the wood and in the primary infection sites. Phytopathology, 102, 827–840.
Ritpitakphong, U., Falquet, L., Vimolmest, A., Berger, A., Metraux, J.P. and L'haridon, F. (2016) The microbiome of the leaf surface of Arabidopsis protects against a fungal pathogen. New Phytol. 210, 1033–1043.

Robert-Seillaniantz, A., Grant, M. and Jones, J.D. (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate–salicylate antagonism. Annu. Rev. Phytopathol. 49, 317–343.

Romling, U., Galperin, M.Y. and Gomelsky, M. (2013) Cyclic d-GMP: the first 25 years of a universal bacterial second messenger. Microbiol. Mol. Biol. Rev. 77, 1–52.

Ross, P., Weinhause, H., Aloni, Y., Michaeli, D., Weinberger-Ohana, P., Mayer, R., Braun, S., de Vroom, E., van der Marel, G.A., van Boom, J.H. and Benziman, M. (1987) Regulation of cellulase synthesis in Acetobacter xylinum by cyclic diguanylic acid. Nature 325, 279–281.

Roux, B., Bolot, S., Guy, E., Denance, N., Lautier, M., Jardinaud, M.F., Fischer-Le Saux, M., Portier, P., Jacques, M.A., Gagnevin, L., Pruvost, O., Lauber, E., Arlat, M., Carrère, S., Koebnik, R. and Noé, L.D. (2015) Genomics and transcriptomics of Xanthomonas campestris species challenge the concept of core type III effectome. BMC Genomics. 16, 1–19.

Ryan, R.P., Fouhy, Y., Lucey, J.F., Crossman, L.C., Spiro, S., He, Y.W., Zhang, L.D., Heeb, S., Camara, W., Williams, P. and Dow, J.M. (2006) Cell–cell signaling in Xanthomonas campestris involves an HD-GYP domain protein that functions in cyclic d-GMP turnover. Proc. Natl. Acad. Sci. USA. 103, 6712–6717.

Ryan, R.P., McCarthy, Y. Andrade, M., Farah, C.S., Armitage, J.P. and Dow, J.M. (2010) Cell–cell signal-dependent dynamic interactions between HD-GYP and GGDEF domain proteins mediate virulence in Xanthomonas campestris. Proc. Natl. Acad. Sci. USA. 107, 5989–5994.

Ryan, R.P., Vorholt, F.J., Potnis, N., Jones, J.B., Van Sluys, M.A., Bogdanove, A.J. and Dow, J.M. (2011) Pathogenomics of Xanthomonas: understanding bacterium-plant interactions. Nat. Rev. Microbiol. 9, 344–355.

Ryffel, F., Helfrich, E.J., Kiefer, P., Peyriga, L., Portais, J.C., Piel, J. and Vorholt, J.A. (2015) Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. GME J. 10, 632–643.

Sarris, P.F., Skandalis, N., Kokkinidis, M. and Panopoulos, N.J. (2010) In silico analysis reveals multiple putative type VI secretion systems and effector proteins in Pseudomonas syringae pathovars. Mol. Plant Pathol. 11, 795–804.

Sauer, A.V., Rocha, K.R., Pedro, E.D., Meirelles, W.F. and Paccola-Meirelles, L.D. (2014) Ice nucleation activity in Pantoaea anaerobia obtained from maize white spot lesions. Semin-Cienc. Agrar. 35, 1659–1666.

Schuster, M., Lostroh, C.P., Ogi, T. and Greenberg, E.P. (2000) A two-component system involving an HD-GYP domain protein links cell–cell communication signal with cross-kingdom structural analogues. Mol. Microbiol. 39, 903–912.

Webber, T., Blin, K., Duddela, S., Krug, D., Kim, H.U., Brucoleri, R., Lee, S.Y., Fischbach, M.A., Muller, R., Wohlbieß, W., Breitling, R., Takano, E. and Medema, M.H. (2015) antiSMASH 3.0—a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res. 43, W237–W243.

White, F.F., Potnis, N., Jones, J.B. and Koebnik, R. (2009) The type III effectors of Xanthomonas. Mol. Plant Pathol. 10, 749–766.

Win, J., Chaparro-Garcia, A., Belhaj, K., Saunders, D.G.O., Yoshida, K., Dong, S., Schornack, S., Zipfel, C., Robatzek, S., Hogenhout, S.A. and Kamoun, S. (2012) Effector biology of plant-associated organisms: concepts and perspectives. Cold Spring Harb. Symp. Quant. Biol. 77, 235–247.

Wonsor, G.L., Lam, D.K., Fleming, L., Lo, R., Whiteside, M.D., Yu, N.Y., Hancock, R.E. and Brinkman, F.S. (2011) Pseudomonas genom database: improved comparative analysis and population genomics capability for Pseudomonas genomes. Nucleic Acids Res. 39, D596–D600.

Wise, A.A. and Binns, A.N. (2015) The receiver of the Agrobacterium tumefaciens VirA histidine kinase forms a stable interaction with VirG to activate virulence gene expression. Front. Microbiol. 6, 1546.

Wu, Z., Kan, F.W., She, Y.M. and Walker, V.K. (2012) Biofilm, ice recrystallization inhibition and freeze–thaw protection in an epiphyte community. PloS. Biokhim. Microbiol. 48, 403–410.

Xin, X.F. and He, S.Y. (2013) Pseudomonas syringae pv. tomato DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annu. Rev. Phytopathol. 51, 473–498.

Yaruyra, P.M., Conforte, V.P., Malamud, F., Roeschlin, R., de Pino, V., Castagnaro, A.P., McCarthy, Y., Dow, J.M., Marano, M.R. and Vojnov, A.A. (2015) XmrX, a new transcription factor involved in the regulation of chemotaxis, biofilm formation and virulence in Xanthomonas citri subsp. citri. Environ. Microbiol. 17, 4164–4176.

Yu, J., Penalvoa-Vazquez, A., Chakrabarty, A.M. and Bender, C.L. (1999) Involvement of the exopolysaccharide alginate in the virulence and epithelial fitness of Pseudomonas syringae pv. syringae. Mol. Microbiol. 33, 712–720.

Yu, X., Lund, S.P., Scott, R.A., Greenwald, J.W., Records, A.H., Nettleton, D., Lindow, S.E., Gross, D.C. and Beattie, G.A. (2013) Transcriptional responses of Pseudomonas syringae to growth in epiphytic versus apoplastic leaf sites. Proc. Natl. Acad. Sci. USA. 110, E425–E434.

Zeng, W. and He, S.Y. (2010) A prominent role of the flagellin receptor FLAGELLIN-SENSING1 in mediating stomal response to Pseudomonas syringae pv tomato DC3000 in Arabidopsis. Plant Physiol. 153, 1188–1198.

Zhou, L., Wang, X.Y., Sun, S., Yang, L.C., Jiang, B.L. and He, Y.W. (2015) Identification and characterization of naturally occurring DSF-family quorum sensing signal turnover system in the phytopathogen Xanthomonas. Environ. Microbiol. 17, 4646–4658.

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