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REVIEW

Against friend and foe: Type 6 effectors in plant-associated bacteria

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Bacterial secretion systems play critical roles in communication with neighboring bacteria and in the modulation of host immune responses *via* the secretion of small proteins called effectors. Several secretion systems have been identified and these are denoted types I–VII. Of these, the type VI secretion system (T6SS) and its effectors were only recently elucidated. Most studies on the role and significance of the T6SS and its effectors have focused on human pathogens. In this review, type 6 effectors from plant-associated beneficial and pathogenic bacteria are discussed, including effectors from *Agrobacterium tumefaciens*, *Dickeya dadanti*, *Rhizobium leguminosarum*, *Pectobacterium atroseptium*, *Ralstonia solanacearum*, *Pseudomonas syringae*, *Pseudomonas fluorescens*, and *Pseudomonas protegens*. Type 6 effectors act in symbiosis, biofilm formation, virulence, and interbacterial competition. Understanding the impact of type 6 effectors on pathogenesis will contribute to the management of bacterial pathogens in crop plants by allowing the manipulation of intra and inter-specific interactions.

Keywords: type VI secretion system, biofilm, pathogenesis, effector, PGPR

Introduction

Bacteria exist in almost all environments, even those with harsh conditions, indicating their ability to adapt in response to their surroundings (Whitman *et al.,* 1998). As well as free-living saprophytic bacteria, certain species of bacteria dominate on and inside eukaryotic organisms and interact with one another and with the host (Coleman, 2001; Akira *et al.*, 2006). These interactions can be classified as beneficial, mutualistic, or harmful with respect to the host. During such interactions, bacteria face competition from organisms of the same domain (Bacteria) and from different domains (Eukarytota and Archaea) (Coleman, 2001; Agrios, 2008). Bacteria utilize their own resources and those of their host in order to successfully occupy their specific niches (Whitman *et al.*, 1998). Bacterial mechanisms that improve survival continue to be discovered, such as those that inhibit the competitor bacterial growth and those that allow the evasion of host immune systems, and these mechanisms have provided new insights into host-bacterial and interbacterial interactions. Bacterial competition strongly affects nutrient uptake through the formation of metabolically interdependent and dependent interactions (Finlay and Falkow, 1989).

Upto date, plant pathogenic bacterial group have extensively studied type I, II, III, and IV secretion systems due to relationship with virulence. Type I secretion system conferring ATP binding cassette (ABC) type transporters is common to general plant pathogenic bacteria that secret bacterial virulence factors (Agrios, 2008). The Sec system to constitute the Sec Y-E-G complex belonging to Type II secretion system resemble to bacterial flagellar basal body to inject mostly hydrolysis enzymes into plant cell (Nivaskumar and Francetic, 2014). Type III secretion system is well-characterized in *Pseudomonas syrinage* that translocate effector proteins and modulates plant immunity (Jin *et al.*, 2003). Type IV secretion system (T4SS) well-defined in *Agrobacterium tumefaciens*, a casual pathogen of crown-gall disease was utilized to deliver DNA from the bacterium into plant cells (Alvarez-Martinez and Christie, 2009; Nester, 2015). However type VI secretion system was only recently evaluated on plant associated bacteria.

General overview of Type VI secretion system

The type VI secretion system (T6SS) was first identified in the opportunistic cystic fibrosis human pathogen *Pseudomonas aeruginosa* in 2006 (Mougous *et al.*, 2006). At the same time, a similar secretion system was identified from a *Vibrio cholerae/Dictyostelium* infection model (Pukatzki *et al.*, 2006). This system was later shown to act as an injectisome for the delivery of secreted proteins into the host cell and was revealed to be T6SS (Pukatzki *et al.*, 2007). Recent genome sequencing of a number of Gram-negative bacteria from plant and animals shed light on the T6SS system and its putative effector proteins (Silverman *et al.*, 2012). Comparative genomic analysis and mutational validation indicated that T6SS was needed for full virulence in animal-pathogenic bacteria (Blondel *et al.,* 2009; Boyer *et al.,* 2009; Sarris *et al.,* 2010). Type 6 effectors in these animal pathogens facilitated the hijacking of host defense responses, stimulated bacterial multiplication, and increased fitness in the presence

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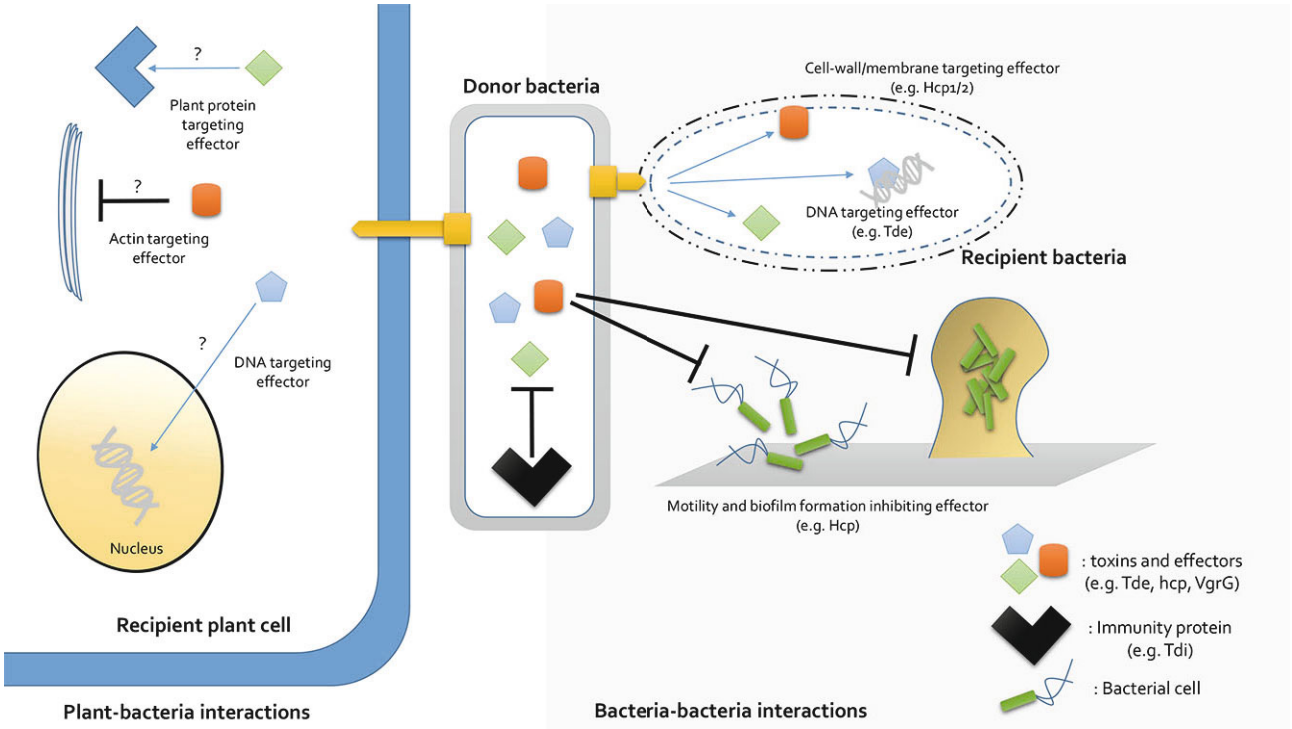

Fig. 1. Schematic depiction of bacterial and host cell-targeting effectors in plant-associated bacteria. Different effector proteins are represented by different shapes. Additional representative type 6 effectors are listed and are discussed in detail in the text. Hcp and VrsG are postulated to target the cell wall, cell membrane, and DNA of competitor bacterial cells (Haapalainen *et al*., 2012; Decoin *et al*., 2014). One toxin (Tse2) inhibited bacterial motility and biofilm formation, resulting in attenuation of virulence (Whitney *et al.,* 2013). Effectors are known to increase plant susceptibility (Mattinen *et al*., 2008; Bröms *et al*., 2013), but the subcellular compartments (e.g., cytosol, periplasm, and outer membrane) that are targeted by specific effectors are not known. Effector toxin and immunity protein pairs increase bacterial fitness by preventing self-toxicity of the effector toxin.

of other bacteria (Schwarz *et al*., 2010; Russell *et al*., 2014). These observations suggested that the T6SS improved bacterial robustness and thereby increased virulence. The exact mechanisms by which bacteria inhibit the growth of other species in the vicinity were only recently understood (Russell *et al*., 2014). Studies on animal-pathogenic bacteria demonstrated that the secretion system played an important role in the dispersion of toxins critical for interbacterial competition (Russell *et al*., 2011; Zhang *et al*., 2013). Recent research revealed that the T6SS is used to transport effector proteins that lack a signal sequence (Schwarz *et al*., 2010). The VgrG (valine-glycine repeat) and haemolysin-coregulated (Hcps) proteins were shown to be the major proteins secreted via this system (Pukatzki *et al*., 2006; Russell *et al*., 2014). VgrG-like effectors can assemble into a trimeric complex, similar to the tail spike of bacteriophage T4, which is involved in puncturing the host cell membrane. Similarly, Hcps generate hexameric ring structures that form a channel for transporting macromolecules into other organisms (Mougous *et al*., 2006).

Although it is now understood that many animal bacterial species, such as *Serratia marcescens*, *Vibrio cholera*, and *P. aeruginosa*, are well equipped for the translocation of effector proteins to other bacteria through the T6SS, little is known regarding the functions of the T6SS in plant-associated bacteria. However, the importance of T6SS activity in natural

Table 1. Roles of Type 6 effectors as antibacterial competitors and plant virulence factors in plant-associated bacteria

Effectors	Activity	Target protein/ Immunity protein	Donor bacterial species	Recipient host plants or bacteria	Phenotype(s)/ targets	References
Virulence/Symbiosis						
Hcp	Unknown	TssB	*Ralstonia solanacearum*	Tomato	Wilt	Bröms *et al.* (2013)
Hcp/VgrG	Unknown	VasK	*Pectobacterium atrosepticm*	Potato	Soft rot	Mattinen *et al.* (2008)
RbsB	ABC transporter		*Rhizobium leguminosaum*	Pea	Nodulation	Bingle *et al.* (2008)
Interbacterial competition						
Hcp1/2	Unknown		*Pseudomonas syringae* pv. tomato	*E. coli and Cryptococcus*		Haapalainen *et al.* (2012)
Tse2	DNase	Tdi	*Agrobacterium tumefaciens*	*E. coli and P. aeruginosa*	DNA	Ma *et al.* (2014)
RhsAB	DNase		*Dickeya dadanti*	*B. subtilis*	DNA	Koskiniemi *et al.* (2013)
Hcp/VgrG	Unknown		*Pseudomonas fluorescens*	*P. atrosepticum*	Peptidoglycan	Decoin *et al.* (2014)
Tge2	Hydrolase	Tgi2	*Pseudomonas protegens*		Peptidoglycan	Whitney *et al.* (2013)

communities remains particularly unclear (Hood *et al*., 2010; Russell *et al*., 2011; English *et al*., 2012; Dong *et al*., 2013a, 2013b).

Examples of toxins secreted from plant-associated bacteria, and their functions, will be discussed in this review. It is important to note that it is not yet known to what extent T6SS-mediated antagonism assists competition between individual bacteria at the inter- and intra-species levels. The diverse roles that the T6SS might play in mediating bacterial interactions with host plants will also be discussed, with a focus on the possibilities suggested by the activities of known effector proteins on plant immune responses and bacterial susceptibility. I exclude any papers conferring *in silico* analysis but include experimentally proved results. The features of the T6SS will also be discussed in plant-beneficial bacteria with respect to potential applications as biological control agents against pathogenic bacteria.

Virulence effector against foe: Another silver bullet to overcome plant defenses

Plant pathogenic bacteria include a large and diverse group of species capable of infecting host plants. Most bacteria cause disease in a single or limited number of host species (Lim *et al*., 2014). The virulence factors from bacteria have been extensively studied and revealed many general virulence factors like toxins and more specific factors like effector proteins involving in the export of proteinaceous factors through secretion systems (Jones and Dangl, 2006). Here, I review the effectors secreted by T6SS out of diverse secretion systems in plant pathogenic bacteria. (Fig. 1 and Table 1)

Pectobacterium atrosepticum

Pectobacterium atrosepticum, which is currently classified as a subspecies of *P. carotovorum*, previously *Erwinia carotovora* (Hauben *et al.,* 1998), is one of a number of bacteria known as pectolytic erwinias that are responsible for soft rot in plants such as cabbage, carrot, tobacco, cotton, cyclamen, cucumber, maize, potato, and delphinium (Thomson *et al.,* 1981). The major virulence factors are plant cell-well degrading enzymes (PCWDE) such as pectate lyase isozymes (Pels), cellulase (Cel), polygalacturonase (Peh), and protease (Prt) (Barras *et al.,* 1994). In addition to these virulence factors, recent comparative genome analysis found more than 20 genes at three loci in the *P. atrosepticum* genome (ECA 3420–3445, ECA2866–2869, and ECA4275–4278) with similarity to known T6SS genes (Schell *et al.,* 2007). Proteomic analysis of bacteria stimulated with host extracts found two effector proteins, Hcp and VgrG (Mattinen *et al.,* 2007). Transcriptional analysis demonstrated that *hcp,* three *vgrG* genes, and T6SS cluster genes were upregulated in the presence of potato tuber extract, indicating that T6SS and its effectors were functionally associated during the interaction of *P. atrosepticum* with its host plant (Mattinen *et al.,* 2008) (Table 1). This resembled the interactions of the human pathogen *V. cholera* strain V52, in which virulence associate secretion (Vas) K was required for Hcp secretion (Das *et al.,* 2002). Analysis of the secretome showed that *V. cholerae vasK* mutants lacked Hcp secretion. These results indicated that VasK could act as a critical mediator of Hcp secretion in *P. atrosepticum* (Mattinen *et al*., 2007). However, *P. atrosepticum* cells carrying *vasK* mutations did not lose their ability to induce PCWDE-mediated soft-rot symptoms and, surprisingly, soft-rot induction was higher in mutants than in wild-type bacteria. Further study showed that growth rate was higher in the mutant than in the wild type, and this led to a higher cell density and an increase in PCWDE production. By contrast, a mutation in one of the seven *P. atrosepticum hcp* homologs led to a slight reduction in virulence. This supported the hypothesis that the Hcp effector was involved in bacterial virulence (Mattinen *et al.,* 2007). The pathways linking VasK-related T6SS with the secretion of effector proteins are unknown, and further experiments will be needed to understand the exact mechanisms underlying *P. atrosepticum* virulence.

Ralstonia solanacearum

R. solanacearum is recognized as one of the most destructive plant-pathogenic bacteria due to its widespread occurrence and broad host range that includes hot pepper, tomato, potato, and tobacco (Agrios, 2008). Full pathogenesis is dependent on the production of extracellular polysaccharides (EPS), cell appendages, and protein secretions. A gene cluster (TssBRS) encoding TssB, one of the hallmark T6SS proteins from human pathogens, was found in the *R. solanacearum* GMI1000 genome (Zhang *et al.,* 2014). The *R. solanacearum* secretion system was validated through mutagenesis studies of the target effectors. Mutation of the target gene cluster led to defective biofilm formation and motility: both of these are key factors for bacterial virulence. Biofilms are aggregates of bacterial cells embedded in a matrix of EPS. Biofilms, which help bacteria survive under stressful environmental conditions, can attach to biotic and abiotic surfaces and can form three-dimensional structures (Davey and O'Tool, 2000; Morris and Monier, 2003; Um *et al*., 2013). Plant-pathogenic bacteria often form biofilms on the plant root surface, and *R. solanacearum* is no exception (Yao and Allen, 2007). *R. solanacearum* is the first plant-pathogenic bacterium in which biofilm formation has been linked to the T6SS. By contrast, the T6SS has been linked to biofilm formation in a number of diverse human pathogens such as *Vibrio parahaemolyticus, E. coli*, and *P. aeruginosa* (Sauer *et al.,* 2002; Enos-Berlage *et al.,* 2005; Southey-Pillig *et al.,* 2005; Aschtgen *et al.,* 2008)

When *R. solanacearum* lacking a functional TssBRS gene cluster was used in plant inoculation experiments, disease index was reduced and bacterial multiplication and colonization within plant tissues were lowered compared to wild type (Zhang *et al*., 2014). Pathogenicity was recovered in a complemented bacterial strain. These results resemble observations in *V. cholerae*, in which VipA (a homolog of TssB) was required for Hcp effector secretion (Bröms *et al*., 2013) (Table 1). TssB therefore plays a fundamental and direct role in the pathogenesis of *R. solanacearum* rather than acting via antibiotic killing capacity. Two components of T6SS in *P. aeruginosa,* TssB and TssA, combine to form a bacteriophage-like tail with a sheath-like tube structure (Lossi *et al*., 2013). Collectively, above results related to virulent function on the effectors from *R. solanacearum* including mutational

and complementation study and the structural study regarding the tail formation resemble observations from previous studies on *Burkholderia mallei* virulence in hamsters (Schell *et al*., 2007) and *V. chlolerae* pathogenesis in amoeba (Bröms *et al*., 2013). The mechanism by which TssB contributes to antibacterial killing of competitor bacterial cells remains to be addressed.

Killing toxin against friend: New weapon against neighboring bacteria

Plant-pathogenic bacteria
Agrobacterium tumefaciens

In 2014, Ma and colleagues described a novel class of nucleic acid-targeting type 6 effectors from the soil bacterium *Agrobacterium tumefaciens* (Ma *et al*., 2014). *A. tumefaciens* triggers crown-gall disease by naturally delivering DNA from the bacterium into plant cells via a type IV secretion system (T4SS) (Alvarez-Martinez and Christie, 2009). Previous transcriptional and posttranslational research in *A. tumefaciens* demonstrated that the T6SS gene cluster was expressed during bacterial infection and in response to acidic plant conditions despite having no direct effect on pathogenesis (Wu *et al.,* 2008, 2012; Lin *et al.,* 2014).

Ma and colleagues discovered a family of type VI DNase effectors (Tde) that demonstrated bacterial killing capacity (Ma *et al.,* 2014) (Fig. 1 and Table 1). An immunity protein, Tdi, was able to detoxify Tde. Tde activity was dependent on a conserved HxxD motif that could be targeted by the Tdi protein. The presence of both Tde and Tdi proteins in *A. tumefaciens* at the plant root promoted bacterial fitness (Wu *et al*., 2008, 2012). By contrast, root-associated bacteria that lacked Tdi were eliminated in the presence of Tde-harboring bacteria. Comparative genomic analysis showed that the tandem gene for the Tde toxin and Tdi immunity protein was conserved in plant-related bacterial genomes (Shneider *et al*., 2013; Ma *et al*., 2014). This suggested that the combination of toxin and immunity protein was fundamental to niche establishment in the microbiome. A similar observation was noted in the human pathogen *V. cholera* during intestine colonization (Fu *et al*., 2013). Research showed that *V. cholera* cells secreted an effector (VgrG3) that targeted peptidoglycans in other bacteria and aided retention in the intestine (Fu *et al*., 2013). This demonstrates that cognate immunity is important for bacterial populations and their interactions in both plant and human environments.

Pseudomonas syringae pv. tomato

P. syringae is a well-characterized plant-pathogenic bacterium that affects a wide variety of plant species (Morris *et al*., 2013). More than 50 pathovars have been reported, and these cause disease symptoms such as spotting, specking, blight, and gall on a variety of crop species (Agrios, 2008). While *P. syringae* is primarily recognized as a foliar pathogen, the bacterium is not limited to leaves and shoots and can also infect plant roots (Morris *et al*., 2013). Many *P. syringae* pathovars translocate virulence effectors into the plant cytosol via the type III secretion system (Jin *et al*., 2003). Effectors play critical roles in circumventing plant defense mechanisms and in host susceptibility. Effector functions are therefore of great interest to researchers interested in understanding bacterial pathogenesis and managing virulence. Partly because of its virulence on *Arabidopsis*, *P. syrinage* pv. tomato DC3000 has emerged as a model plant-pathogenic bacterium for the investigation of plant-bacteria interactions (Xin and He, 2013). Several effector-encoding hypersensitive response and pathogenicity (*Hrp*) and Hrp outer protein (*Hop*) genes have been discovered, mostly in *P. syringae* pv. tomato DC3000, through *in silico* and biological screening methods (Schechter *et al*., 2006; Cunnac *et al*., 2009; Schumacher *et al*., 2014). The major roles of effectors are to suppress plant defenses and stimulate bacterial multiplication in host tissues (Schechter *et al*., 2006; Cunnac *et al*., 2009). The specific plant metabolites that elicit bacterial virulence factors are poorly understood. Haapalainen and colleagues used tomato cell extracts as part of a Hrp-inducing medium in a proteomic study to identify novel secreted components (Haapalainen *et al*., 2012) (Fig. 1 and Table 1). Hcp, an extracellular component of the T6SS, was identified in this experiment. Genome analysis of *P. syringae* pv. tomato DC3000 revealed two *hcp* genes: *hcp1* (PSPTO_2539) and *hcp2* (PSPTO_5435). The two genes are expressed dissimilarly in plant tissues. The *hcp1* gene is null functional, but the HCP2 protein is secreted via the T6SS and appears as a covalently combined dimer *in vitro*. By contrast with the roles of Hcp proteins in animal pathogenesis, HCP2 in plant-pathogenic bacteria does not contribute to infection and colonization activity but is necessary for persistence and for successful competition with yeasts and with other bacteria. These novel results demonstrated that type 6 effectors in *P. syringae* were important for fitness and allowed the pathogen to sustain colonization when competitively challenged for food resources by other microflora in the same niche.

Dickeya dadantii

Dickeya dadantii (*Erwinia chrysanthemi*) is a phytopathogenic bacterium that causes soft-rot diseases in many different crops (Hugouvieux-Cotte-Pattat and Condemine, 1996; Ma *et al*., 2007). In *D. dadantii*, two rearrangement hotspot (Rhs)/related YD repeat-containing proteins are delivered by the T6SS (RhsA and RhsB). These effectors carry C-terminal nuclease domains that degrade target cell DNA (Koskiniemi *et al*., 2013) (Fig. 1 and Table 1). *D. dadantii* 3937 *rhs* genes do not encode secretion signal sequences but are instead linked to the Hcp and VgrG system; this indicates that effector secretion occurs via the T6SS. The detail function of the candidate effect need to be proven in near future.

Plant-beneficial bacteria
Rhizobium leguminosarum

Plant associated bacteria contribute to plant growth and health and those bacteria coined as plant-beneficial bacteria (Ryu, 2013; Ryu *et al*., 2013). Although not much were known about the role of T6SS in plant beneficial bacteria, there are several example of T6Ss in plant-beneficial bacteria playing

a role for plant-microbe interaction (Bingle *et al*., 2008; Boyer *et al*., 2009). T6SS and effectors have been studied in plant-beneficial bacteria as well as in pathogenic and saphrophytic species. The T6SS and its effect on root nodulation was reported in symbiotic bacteria such as *Rhizobium* spp., *Mezorhizobbium* spp., and *Shinorhizobium* spp. In 1997, Roest and coworkers isolated a novel gene cluster from a strain of *Rhizobium leguminosarum* bv. *trifolii* that prevented optimal nodule formation and function on pea roots (Roest *et al*., 1997). The locus, which did not share similarity with any known genes, was later termed impaired in nodulation (*imp*) (Bingle *et al*., 2008; Boyer *et al*., 2009) (Fig. 1 and Table 1). In 2003, a 2DE proteomic approach was used to determine the Imp-dependent protein secretome. This resulted in the discovery of a 27-kDa protein, RbsB, that was produced by one of the genes in the *imp* cluster (Bladergroen *et al*., 2003).

The discovery of the *imp* cluster in *R. leguminosarum* has parallels with work in *Vibrio cholerae*. A protein homolog, IcmF, was identified and the corresponding *icmF* gene was isolated from an experiment using a rabbit infection model (Das *et al.,* 2002; Das and Chaudhuri, 2003). Further mutant screening with *V. cholerae* in an amoeboid *Dictyostelium discoideum* model identified 'virulence associate secretion' (*vas*) genes. One of these genes, *vasK* (VAC0120), matched the known *icmF* (Das *et al*., 2002; Bladergroen *et al*., 2003). Collectively, these data suggested that the *imp* and *vas* genes encoded proteins involved in plant infection and amoeboid killing, respectively, and that these systems were highly similar. The another effectors from *R. leguminosarum* was studied and denomstrated (Bladergroen *et al*., 2003). RbsB proved to be a small ribose-binding protein that was translocated through the T6SS and played a role in nitrogen fixation (Bladergroen *et al*., 2003). Protein alignments showed that RbsB was similar to an autoinducer-2 (AI-2)-binding protein, LuxP, from *V. harveyi*. RbsB from *Actinobacillus actinomycetemcomitans* exhibited binding activity with AI-2, which suggested a putative role for RbsB in the regulation of quorum sensing (Shao *et al.,* 2007). However, the detailed biological roles of RbsB in plant-associated bacteria, including rhizobia, remain to be elucidated.

Pseudomonas protegens

Recent *in silico* genomic studies of the soil bacterium *Pseudomonas protegens* identified Tge2-Tgi2 (effector toxin and immunity protein respectively) from a search of type 6 effector immunity genes located adjacent to effector genes (Whitney *et al*., 2013) (Fig. 1 and Table 1). Crystal structure analysis of the effector showed that it resembled a glycoside hydrolase family protein that was associated with peptidoglycan N-acetylglucosaminidase activity. This suggested that the T6S peptidoglycan glycoside hydrolase effector families may represent significant enzymatic diversity. The immunity protein Tgi2 abrogated the activity of Tge2 in *P. protegens* by direct occlusion of the effector active site. Bacteria in the neighboring environment that lacked the *tgi*2 gene were killed, and this conferred a competitive advantage upon *P. protegens.*

Pseudomonas fluorescens

In addition to investigating the T6SS features of *P. syringae*, Decoin and coworkers also observed *in vitro* secretion of T6SS effectors from a non-pathogenic and plant growth-promoting rhizobacterium (PGPR) *Pseudomonas fluorescens* strain MFE01 (Decoin *et al*., 2014) (Fig. 1 and Table 1). The secreted proteins included Hcp and VgrG. The two effectors are expressed in a range of bacteria and act as toxins against competitor bacteria and eukaryotic cells. In human pathogenic bacteria such as *Vibrio cholerae*, *Burkholderia* spp., and *P. aeruginosa*, this is mediated by direct injection of the effectors into the cytosol (Ho *et al*., 2013, 2014). The production of these two effectors in a saprophytic bacterium was therefore unexpected. Functional mutational analysis of putative *hcp* homologs in the MFE01 genome (*hcp1* and *hcp2*) revealed no virulent function against eukaryotic cells but did demonstrate antibacterial killing capacity against a broad spectrum of pathogenic bacteria including *E. coli*. The bacterial killing capacity of strain MFE01 against *E. coli* cells was abrogated by the inclusion of an immunity protein from *S. marcescens,* indicating that the type 6 effector target was peptidoglycan on the Gram-negative cell surface (English *et al.,* 2012; Russell *et al.,* 2014). Strain MFE01 was used effectively as a pretreatment to protect potatoes against soft-rot symptoms caused by *P. astrosepticum*. MFE01 mutants lacking Hcp2 were unable to confer this protection.

Conclusions and future perspectives

Many of the recent advances in our understanding of the T6SS have been made through the identification and description of T6SS components and secreted effectors. The effectors identified to date exhibit antibacterial activity towards susceptible recipients. This highlights the importance of the T6SS in mediating interbacterial interactions and plant-bacterial interactions. This review discusses T6SS and their effector proteins in plant-associated bacteria and the influence of these factors on bacterial fitness, virulence, and competence on interaction with other organisms (Fig. 1). In many instances, the T6SS provides bacteria with mechanisms to compete effectively in microbial communities or host environments. Many aspects of type 6 effector secretion, including signal cues, signaling pathways, and regulation in donor bacteria, remain poorly characterized (Fig. 1). Investigations of the regulation of effector secretion in donor bacteria should ultimately provide new insights into the molecular targets of antibacterial compounds. Screening and identification of inhibitors that attenuate function of the secreted effectors will provide new control methods to combat infection by decreasing the ability of pathogens to compete against neighborhood microflora.

Understanding the precise mechanisms underlying effector recognition and downstream pathways in recipient bacteria is important for the understanding and management of pathogenic bacteria. Increasing our comprehension of the nature and role of type 6 effectors in the context of bacterial interactions with plants and competitor bacteria may also shed light on novel functions of bacterial secreted proteins. Many questions remain to be addressed, including: 1) Which

plant proteins are targeted by type 6 effectors once the effectors are translocated into the host cytosol?; 2) Are environmental and host signals involved in T6SS initiation?; 3) Are type 6 effectors present that target both bacteria and plant cells?; 4) Do plants produce immunity proteins against type 6 effectors?; 5) How is the secretion of type 6 effectors regulated when the T6SS secretes multiple effectors? (Fig. 1).

The question of how bacteria perceive and respond to single or multiple effectors could be addressed using large-scale mutagenesis screens based on T6SS-dependent responses, as well as by transcriptomic, proteomic and metabolomic analyses.

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