Asif *et al. Phytopathology Research* (2025) 7:28
https://doi.org/10.1186/s42483-024-00304-2

Phytopathology Research

REVIEW

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# Regulation and inhibition of type III secretion systems in plant pathogenic bacteria

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## Abstract

Type III secretion systems (T3SS) are syringe-like apparatuses acting as protein transport nanomachines found in most Gram-negative bacterial pathogens. They can inject effector proteins into the host cell cytoplasm, crossing the host cell membrane, and cause infection. Due to their critical role in pathogenicity, T3SS represent attractive targets for vaccinations and disease treatments. This review elucidates the overarching structural framework and operational mechanisms of T3SS apparatuses while also delineating the responsiveness of phytobacterial T3SS to host-derived signals and the nuanced orchestration of their activities by host and environmental stimuli. This discussion encompasses shared features and idiosyncratic attributes among a spectrum of pathogens, including but not limited to *Pseudomonas syringae*, *Ralstonia*, *Xanthomonas*, and *Erwinia*. Additionally, we scrutinize the contribution of natural products and synthetic chemicals as T3SS inhibitors, elucidating their hallmark and role in the ongoing quest for and design of novel drugs. An in-depth comprehension of T3SS functionality and the modes of action of diverse inhibitors holds promise for developing innovative drugs aimed at swiftly suppressing phyto-pathogenicity elicited by a spectrum of bacterial species.

**Keywords** Type III secretion system, Nanomachines, Regulation, T3SS inhibitors, Natural and synthetic chemicals

## Background

Most pathogens secrete proteins that play a crucial role in mediating the complex interactions with other organisms in their surrounding environment. These pathogenic bacteria are equipped with specialized excretion systems (type I–VII) to accomplish intricate biochemical tasks of transporting proteins across cellular membranes. Among them, the type III secretion system (T3SS) has been extensively studied in various model organisms (McDermott 2011; Notti et al. 2015; Notti Ryan and Stebbins 2016; Deng et al. 2017). These pathogens are primarily associated with various plant and human diseases (Fig. 1). Pathogenic bacterial strains can deploy T3SS to inject effector proteins into the host cell cytosol to modulate myriads of host cell processes and to establish an intracellular or extracellular niche suitable for differentiation, replication, and dissemination, as illustrated (Fig. 1). The successful establishment of pathogens (extracellular or intracellular) depends on the efficient hijacking of the host immune response, functional machinery, and cytoskeleton. The best-known examples include *Escherichia coli* (Entero-pathogenic and Entero-hemorrhagic; EPEC and EHEC), *Citrobacter rodentium, Chlamydia*, *Salmonella*, and *Shigella* strains. Because of such invasions, these pathogens can easily invade hosts cells via T3SS. Humans and animals have suffered dysentery and respiratory infections, leading to epidemics of plagues. Similarly, plant hosts resulted in pandemics of canker, blights, and soft rots (Fig. 1; Abby and Rocha 2012).

A well-documented example is plant-associated *Pseudomonas syringae* and *P. aeruginosa* strains, which could show higher virulence. *P. syringae* have multiple hosts to attack and cause infection by manipulating the plant

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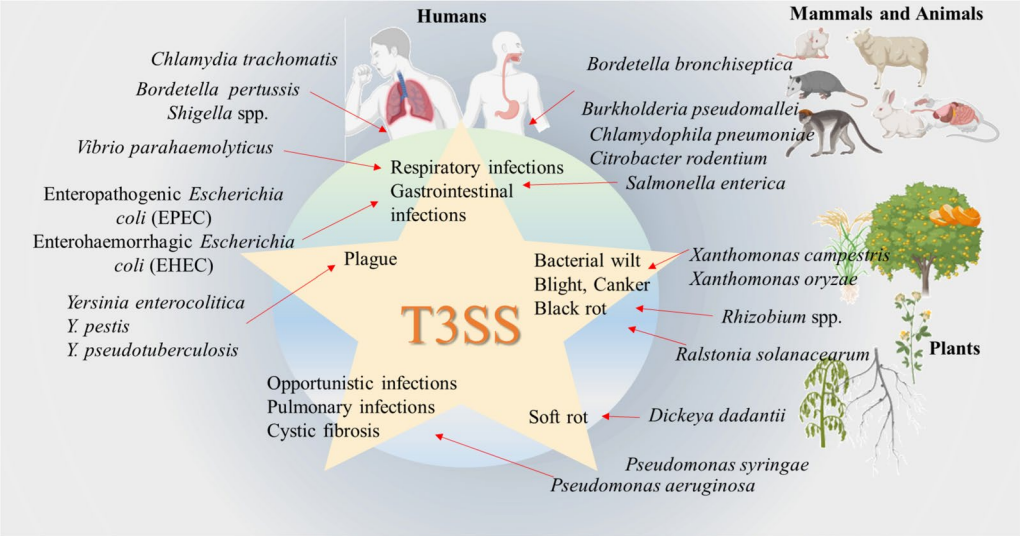



**Fig. 1** Overview of model bacteria endowed with T3SS(s). Particular examples are the pathogenic bacteria identified in humans, mammals, animals, and plants, wildly known to cause epidemics in the world by causing devastating diseases. Data was compiled from Hueck (1998), Abby and Rocha (2012)

defense system (immunity) with its T3SS nanomachine. Similarly, *P. syringae, Xanthomonas*, *Ralstonia solanacearum*, *Rhizobium*, and soft rot-causing agents *Dickeya* and *Pectobacterium* spp., all have diverse hosts and conserved T3SS to suppress the immune response (Davidson et al. 2013, 2017; Landry et al. 2020; Asif et al. 2024b). T3SS relies on a conserved set of genes to function effectively in these strains. Many structural protein-forming nanomachine apparatus are the same in most of the strains as HrcC forms the outer ring of the T3SS apparatus, while HrcJ anchors the structure to the bacterial cell envelope by contributing to the basal body. HrcR and HrcS (HrpS) regulate the expression of T3SS components and the associated effector proteins. HrcU plays a pivotal role in protein secretion and translocation, while HrcV forms the translocon to ensure that effector proteins are delivered into the host cells. HrcN, an ATPase, provides the energy required for protein translocation through this system. Finally, the HrpB and HrpD families control the expression and secretion of the T3SS machinery in specific bacterial species. Together, this network of genes and proteins coordinates for assembly and regulation of the T3SS, allowing pathogenic bacteria such as *P. syringae*, *R. solanacearum*, *Xanthomonas* spp., and *Erwinia* spp. to infect their plant hosts. These pathogens inject their effector proteins that manipulate host cells to promote bacterial survival and colonization (Helmann et al. 2019; Teulet et al. 2022). Despite the diversity of the host, there is remarkable conservation in the T3SS apparatus, secretion mechanisms, and their mode of action (Du 2016; Portaliou et al. 2016; Helmann et al. 2019).

Since its discovery in 1994 (Rosqvist et al. 1994; Coburn et al. 2007), the T3SS has been systematically categorized into five primary components: the translocon, the needle complex, the basal body, the transfer apparatus, and the cytoplasmatic complex (Fig. 2). The translocation mechanism is a critical component of the T3SS, facilitating the transfer of proteins from the bacterial cytoplasm to the host cytoplasm (Büttner 2012, 2016; Burkinshaw and Strynadka 2014; Burkinshaw 2015; Sun et al. 2007), and the delivery unit is called as needle complex. This complex includes an ATPase and multiple circular structures (rings) that are intricately integrated into the membrane (Chang et al. 2014; Bernal et al. 2019; Gupta et al. 2024). Contrary to animal pathogens, plant pathogenic bacteria, such as pathovars of *P. syringae* and *Xanthomonas* spp., possess an elongated needle, referred to as a pilus, which enables penetration of the thick cell wall and contact with the underlying plasma membrane (Bergeron 2013, 2016). Likewise, EPEC, EHEC, and *Citrobacter rodentium* possess a filamentous extension (EspA) that exhibits structural similarities to flagellin (Lindeberg et al. 2006; Xin et al. 2018). Interestingly, the T3SS needles of plant pathogens (termed Hrp pili) have neither a tip complex nor an extension, but are considerably longer than the needles of the T3SS of animal pathogens, probably so that they can penetrate the thick cellulose matrix of the plant cell wall. These needle modifications are likely to be evolutionary adaptations for infecting different hosts. The needle tip protein is directly associated with the transport apparatus (Diepold and Wagner

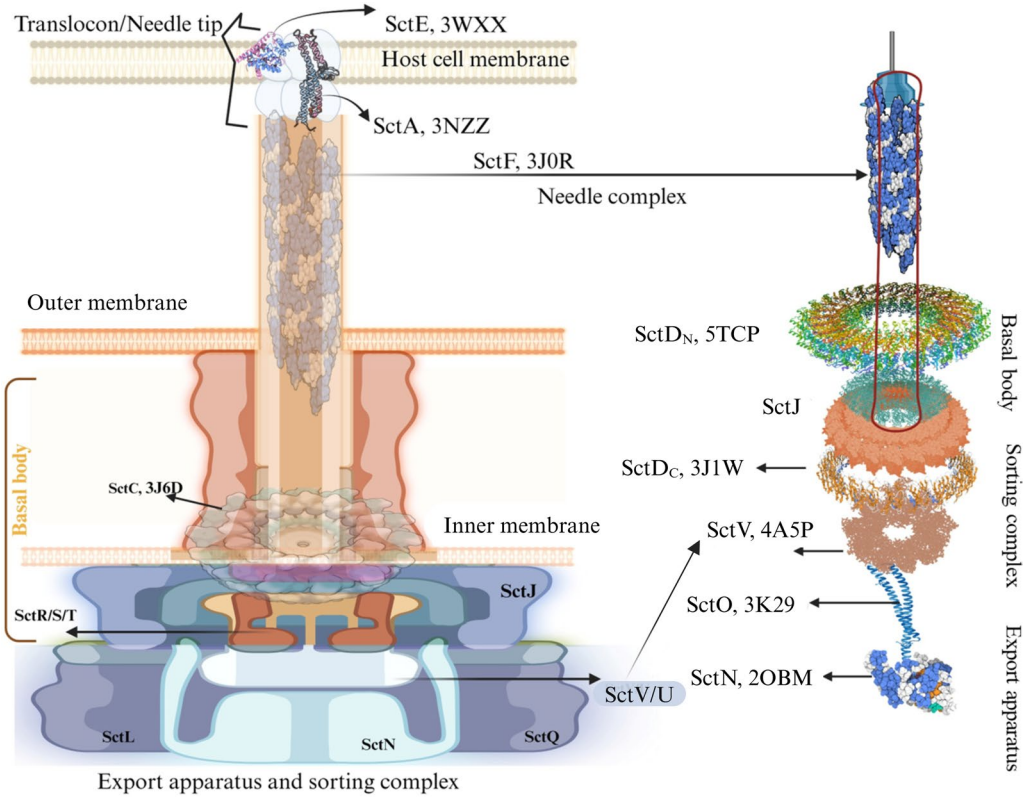



**Fig. 2** The structural and functional illustrative model of T3SS nanomachine in *Salmonella* spp. SPI-1. In *Salmonella* SPI-1 T3SS, the needle complex, basal body, export apparatus, and sorting complex, are shown on the left side. The cross sections of the inner structure of these components are shown on the right side. On the right side, electron microscopy-mediated solved protein structures are compiled to display basic nanomachine orientations. SctC and SctD proteins transfer to the inner rod in the inner membrane and pass to the translocon or needle complex (SctF). Accession numbers from the Protein Data Bank (PDB) were used to search, and carton displays were created in Biorender. The proteins, accession IDs, and their names are listed as Needle complex. SctE (AopB-3WXX; and SipB-3TUL), SctA (SipD; PDB-3NZZ), SctF (PDB-3J0R), SctC; (PDB-5TCQ & 5TCR) (InvG); SctD$_N$ (PDB-3J1W) (InvG), SctD$_C$; (PDB-5TCP), SctJ (PrgH-PrgK::PrgK; PDB-3J6D, – 5TCP; – 5TCR), SctU (SpaS; PDB-3C01, SpaS$_C$), SctV (PDB-4A5P), SctO (PDB-3K29), SctN (PDB-2OBM) (Abrusci et al. 2013; Bergeron et al. 2013; 2015; Chatterjee et al. 2011; Fujii et al. 2012; Lorenzini et al. 2010; Worrall et al. 2008, 2016; Zarivach et al. 2007, 2008; Deng et al. 2017)

2014; Diepold et al. 2015; Tang et al. 2018). The needle is a cylindrical structure composed of helical protein subunits, with its length determined by the host organism of the pathogen (Fig. 2; Lefebre and Galán 2014; Bergeron 2016; Murillo et al. 2016; Tang et al. 2018).

The ongoing research on specific pathogens involves exploring various functions, regulatory pathways, and inhibitors. However, a comprehensive compilation of comparative data to understand commonalities and specificities among pathogens is still lacking. Such data would clarify the interplay between pathogens, hosts, and environmental cues, particularly regarding the response of phytobacterial T3SS to host signals and host and environmental stimuli regulation. Plant pathogens like *Pseudomonas* spp., *Ralstonia* spp., *Xanthomonas* spp., and *Erwinia* spp., are crucial subjects for study. In addition, dedicated efforts are required to identify inhibitors of the T3SS from both natural products and synthetic compounds. These investigations should aim for a comprehensive understanding of their mechanisms of action and specific molecular targets within the T3SS machinery of these pathogens. This review provides a comprehensive analysis of the structural framework of the T3SS apparatus, its architectural associations, co-regulatory mechanisms across various species, and the functional roles of its genes. Additionally, we have examined the functional overlap between distinct two-component systems in the species discussed, identified inhibitory compounds, and elucidated the modes of action of diverse inhibitors with potential for novel drug development. Furthermore, we have conducted comparative studies on pathogen infection dynamics and explored bacterial manipulation strategies across different domains.

**Regulation of T3SS in plant pathogenic bacteria**

In Gram-negative bacteria, T3SS encoded by *hrp*/*hrc* (HR and Pathogenicity; HR and Conserved) cluster is a prevalent feature observed in various genera including *Erwinia, Pseudomonas, Ralstonia*, and others (Alfano et al. 2000; Andrade et al. 2014). The majority of *hrp* genes are situated within chromosomes, forming pathogenic islands, although a subset of *hrp* genes, such as *hrpM* in *P. syringae*, is dispersed throughout the chromosome. Typically, the *hrp* gene cluster size ranges from 18 to 40 kb, encompassing approximately 20–25 genes, among which highly conserved genes are denoted as *hrc* genes. Meanwhile, a functional common nomenclature includes "secretion and cellular translocation" (Sct) as a unified system for conserved components of T3SS for all species is developed, widely adopted and recently expanded (Gazi et al. 2012; Peeters et al. 2013; Diepold and Wagner 2014; Portaliou et al. 2016; Gaytán et al. 2016; Portaliou et al. 2016). The T3SS spans the bacterial inner and outer membranes, and the host cell membrane, comprising several substructures: a cytosolic ATPase complex, C-ring, inner membrane export apparatus, basal body, needle, and translocation pore in the host membrane (Fig. 2). The basal body is composed of inner membrane rings (SctJ, SctD) and an outer membrane ring (SctC), with the export apparatus formed by SctR, SctS, SctT, SctU, and SctV. Below this, the C-ring (SctQ) and ATPase complex (SctN, SctO, SctL, SctK) form the sorting platform for secretion (Schraidt et al. 2010; Schraidt and Marlovits 2011; Burkinshaw and Strynadka 2014; Hu et al. 2015; Makino et al. 2016; Portaliou et al. 2016). The inner rod anchors the needle, which is capped by the SctA tip complex and connects to host cells through translocators SctE and SctB. The length of the needle is regulated by SctP (Journet et al. 2003; Thomas et al. 2012; Wee and Hughes 2015), while SctU and SctW control the secretion hierarchy (Büttner 2012). A functional T3SS, containing the basal body and needle, is referred to as the 'needle complex', analogous to the flagellar hook-basal body structure (Fig. 2; Macnab 2003; Schraidt and Marlovits 2011). Sct hereafter is referred to as conserved *hrp/hrc* locus for *P. syringae* nomenclature. HrcV exhibits significant homology with their counterparts in *Yersinia* (Xin et al. 2018). The T3SS is the main virulence determinant in most pathogenic *Xanthomonas* species, encoded by the conserved *hrp*/*hrc* locus similar to *Pseudomonas* T3SS, having 20 proteins with multiple copies to develop the major T3SS subassemblies: the sorting platform (HrcQ, HrcL, HrcN, and HrpB7), the export apparatus (HrcR, HrcS, HrcT, HrcV, and HrcU), the needle complex (HrcD, HrcJ, HrcC, and HrpE), and the translocon (HrpF) (Alvarez-Martinez et al. 2020).

Based on the genetic similarity within the *hrp* cluster, it consists of two distinct groups (Baltrus et al. 2017; Wang et al. 2018). The first group, "Hrp1", includes *E. amylovora*, *P. syringae*, and *Pantoea* spp. (Baltrus et al. 2017), and the second group, "Hrp2", comprises *R. solanacearum*, *Xanthomonas* spp., and *Acidovorax citrulli* (Clarke et al. 2010; Abby and Rocha 2012). The primary disparity between these two groups lies in their transcriptional regulation systems. The first group, including *P. syringae*, regulates the T3SS expression using extracytoplasmic function sigma (σ) factor *rpoN* and alternative sigma factor *hrpL* transcriptionally (Fig. 3a), while both *Erwinia* spp., and *Pseudomonas* spp., also utilize the upstream transcriptional regulator HrpS alone or together with HrpR to control the Hrp genes expression. In addition, Hrp2 species use the transcriptional regulator HrpR to modulate the HrpS expression, subsequently activating the functional action of *hrpL* to control T3SS activity (Hutcheson et al. 2001; Jovanovic et al. 2014; Wang et al. 2018). In the second group, members of the AraC family of proteins regulate transcriptional activity associated with T3SS (Chang et al. 2014; Costa et al. 2015; Waite et al. 2017).

*R. solanacearum* and *Xanthomonas* spp. synthesize their T3SS using about 20–22 *hrp*/*hrc* genes, which contain tight regulatory control. It is unique in linking T3SS gene expression directly to plant host contact, using effectors to alter host defense and metabolic pathways (Rico and Preston 2008; Xian et al. 2020). Expression of *hrp*/*hrc* genes is triggered when the outer membrane receptor PrhA detects an unidentified cell wall component, initiating a signaling cascade. HrpG, an OmpR-type regulator, activates HrpB, which directly regulates T3SS genes and effectors, likely by binding to the *hrp* box motif (Occhialini et al. 2005; Coll and Valls 2013). HrpG and HrpB have homologs in *Xanthomonas* and *Burkholderia* species (Li et al. 2011; Lipscomb and Schell 2011), but PrhA and its upstream regulators are not conserved in other species. Moreover, the *Xanthomonas* T3SS cluster facilitates the translocation of type III secreted effectors into plant host cells (White et al. 2009). These effectors, known as Xops (*Xanthomonas* outer proteins), are crucial for *Xanthomonas* pathogenicity. The T3SS cluster is uniquely positioned in *X. campestris* pv. *campestris*, suggesting independent acquisition as indicated by its chromosomal location, distinct from other group 2 species (Jacob et al. 2015; Merda et al. 2017). However, further studies related to pathogenicity-related genes in *R. solanacearum* and their mechanisms of regulation are extensively reviewed elsewhere (Peeters et al. 2013; Lowe-Power et al. 2018; Vailleau and Genin 2023).

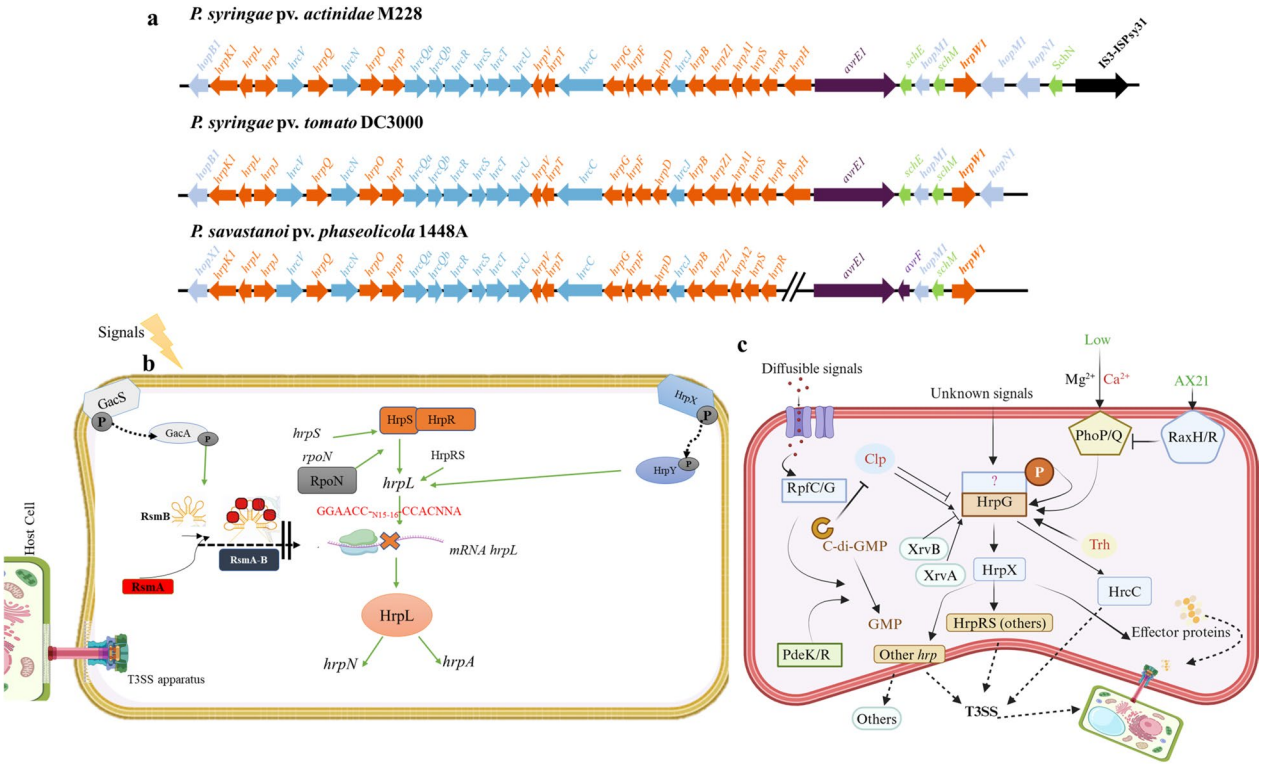



**Fig. 3** The pathogenicity island and basic master regulators of T3SS genes. **a** The pathogenicity islands present in the *Pseudomonas syringae* pv. *actinidae* M228, compared to gene orientation in *P. syringae* pv. *tomato* DC3000 and *P. savastanoi* pv. *phaseolicola* 1448a. This orientation shows the similarity and uniqueness of the three strains and their evolutionary aspects. For the orientation of the T3SS cluster of *P. syringae* pv. *tomato* DC3000 and *P. savastanoi* pv. *phaseolicola* 1448a was adapted from Xie et al. (2019). **b** HrpL master regulator influenced by different factors, especially HrpX (HrpG, V, U), TCS, and RSM system, and ultimately infects the host plant. **c** the master regulator *hrpG* regulated by different sigma factors (PhoPQ) and other factors in *P. aeruginosa*. The display data was compiled from Anantharajah et al. (2016), Xie et al. (2019)

## Transcriptional and post-transcriptional regulation of T3SS genes

The major transcriptional regulatory control of the T3SS cascade is under the HrpL protein belonging to the ECF family. This protein, along with its alternate sigma factor ($\sigma^{54}$), interacts with a conserved "*hrp*-box; GGAACC $^{-}$N15-16-CCACNNA" located in the promoters of certain genes involved in the T3SS regulation (Fig. 3b; Fouts et al. 2002; Xiao et al. 2004; Chang et al. 2014). HrpL facilitates the transcription of the *hrp*/*hrc* and other T3SS effector genes with the assistance of RNA polymerase, resulting in improved pathogenicity (Hutcheson et al. 2001; Zwiesler-Vollick et al. 2002; Schechter et al. 2006; Ferreira et al. 2006; Huang et al. 2022; Zhao et al. 2022). Many studies are inclined to propose that HrpL-mediated regulation and coordination with other T3SS-encoding genes are achieved in a well-organized pattern. It precisely controls the expression and timely induction of associated genes in the pathway (Ferreira et al. 2006; Thwaites et al. 2004; Waite et al. 2017). The interruption of the *hrpL* gene during the natural or synthetic process of evolution results in impaired *hrpL* promoter activity, transcriptional activity, and subsequent base mutation in the promoter region, resulting in the loss of pathogenicity in *P. syringae* pv. *actinidae* (Xie et al. 2023).

In addition to the $\sigma^{54}$ sigma factor (RpoN), two other proteins HrpR and HrpS, also play a crucial role in the transcription of *hrpL* regulation and start the T3SS gene expression (Ortiz-Martín et al. 2010a; Wang et al. 2018). These two proteins are found in a HrpRS locus present inside the *hrp*/*hrc* cluster (Hendrickson et al. 2000; Wang et al. 2018). Moreover, the HrpR is required for *hrpS* transcription, and this interaction with an upstream regulatory sequence known as the Hrp-box was described in halo-blight bacterium NPS3121 (Ortiz-Martín et al. 2010a; Jovanovic et al. 2011). The oligomerization of HrpR and HrpS results in the formation of a heterohexameric complex, essential for the complete activation of the *hrpL* gene. However, it should be noted that HrpS (Hutcheson et al. 2001), alone can interact with *hrpL* promoter to activate it. Still, the potential expression would be weak when the other partner, *hrpR*, is not present (Jovanovic et al. 2011). In addition to the activation of *hrpL*, *hrpS* is responsible for regulating the activation of

several genes that are not dependent on T3SS throughout the entire genome (Lan et al. 2006, 2007; Wang et al. 2018). A study on three deficient mutants of *hrpL*, along with *hrpRS* genes in *P. syringae* DC3000, revealed rapidly accumulated gene expression of different housekeeping genes other than T3SS. This transitional switching off of T3SS activity to a widespread increase of the normal metabolic processes indicated a tradeoff function in *P. syringae* (Lan et al. 2006).

Moreover, HrpA (or HrpA1) is known to form pilus protein and exert its transcriptional regulatory effect on the T3SS pathway (Wei et al. 2000). The *hrpA* gene is located on the upstream region of *hrpRS* and *hrpL* in the *P. syringae* DC3000 and is necessary for the release of different substrates primarily as harpins (Abrusci et al. 2013; Waite et al. 2017). HrpA is a gene regulated by HrpL and can be activated by soluble signals from plant cells (Wei et al. 2005; Waite et al. 2017). HrpA1 is necessary for the induction of *hrpL* and *hrpS* transcription in *P. syringae* pv. *phaseolicola* 1448a in the leaves of host bean plants (Ortiz-Martín et al. 2010b). To activate the HrpZ operon expression, *P. syringae* pv. *phaseolicola* and *syringae* DC3000 show the same upregulated expression of *hrpL*, *hrpRS*, and *hrcC* mRNA transcripts upon *hrpA* mutation compared to wild-type control, upon contact with the plant, suggesting the presence of an important feedback mechanism to control T3SS by *hrpA* (Wei et al. 2000; Ortiz-Martín et al. 2010b). Although HrpA can autoactivate the T3SS pathway, negative self-regulation prevents excessive production of T3SS-related proteins. An additional promoter region containing *hrp*-box is also situated in the *hrpJ* gene, and the promoter activity of *hrpL* is downregulated by HrpL itself through its binding to that box. This HrpL-T3SS signaling cascade exhibits robust signal amplification, while negative autogenous regulation prevents excessive accumulation of T3SS substrates (Waite et al. 2017). Similarly, higher expression of *hrpL* transcripts increased the extracellular accumulation of HrpA1, but the increased level of T3SS harpin and effector proteins were not secreted out of the cell. The intracellular accumulation of these compounds in *P. syringae* modulated the T3SS in two distinct phases: First, HrpL-dependent expression of T3SS genes and secretion of HrpA1, and second, secretion of downstream T3SS substrates, which appears to be regulated post-translationally. The exact mechanism triggering the secretion of these substrates remains unknown, but it is likely linked to the maturation of the HrpA1 and its contact with the host plasma membrane (Waite et al. 2017).

The HrpRS heterodimer, which plays a role in the transcriptional activation of *hrpL*, undergoes translational control by two other small proteins HrpV and HrpG (Fig. 4a). Accordingly, HrpV has a direct interaction with HrpS, resulting in a constraint on the binding capacity of HrpS to HrpR (Jovanovic et al. 2011, 2014). HrpV can interact with HrpG (a chaperone protein) linked to the T3SS basal body located at the plasma membrane (Jovanovic et al. 2011). It is postulated that HrpV binds

(See figure on next page.)

**Fig. 4** The identified factors influencing the de-repression and suppression of T3SS regulation in terms of HrpRS-HrpL pathway in *P. syringae*. **a** HrpL transcription is regulated by different proteins that include AefR, PsrA (indirect positive activators), and CorR upstream binding agent (binds directly to HrpL and activates early transcription). HrpJ established a binding complex with *hrpL* promoter to prevent the formation of HrpRS complex, which creates a negative feedback loop to negatively regulate *hrpL* activity. Then, HrpS protein itself can stimulate the T3SS effector proteins. AlgU and HrpA induce the overexpression of the *hrpRS* locus in a direct manner. In GacSA TCS, phosphorylated-*gacA* binds indirectly to the IR (inverted repeats) region of *hrpR* and *hrpL* to induce their expression. CvsR has a direct regulatory effect on the activation of *hrpRS*, which is expressed in the presence of calcium ion $Ca^{2+}$ as an external environmental stimulus and ultimately sustains the T3SS-dependent virulence in *P. syringae*. In a higher level of c-di-GMP, HrpR/HrpL expression is repressed. In addition, HrpV blockage is removed by HrpG activity and it frees the HrpS as well as HrpJ makes a complex with both (HrpJG/V) to allow the induction of the expression of T3SS. Thus, HrpG is the anti-repressor of an anti-activator HrpV. The bonding of HrpG with HrpF exerts a negative regulatory effect on T3SS by allowing the formation of the HrpRSVG circuit. P-RhpR independently activates T3SS effector genes like *hopR1*. The chaperone protein RhpC directs metalloprotease RhpP to the periplasm. In the absence of RhpC, RhpP decreases the expression of HrpL. Induction of *hrpRS* and *hrpL* transcription requires (p)ppGpp. **b** *P. syringae* cultured in Kings B (nutrient-rich condition, KB) resulting in suppression of its T3SS; *hrpL* gene allows HrpT to bind its promoter and motif which indicates an indirect regulation by HrpT as shown by dotted lines, The PRhpR starts to pile-up and its abundance leads to the suppression of *hrpRS* to check its low expression. HrpV influences the binding affinity of HrpS protein to prevent the oligomerization of HrpRS; HrpRS heterodimer destruction and negative regulation is described with red dotted arrow. Phosphorylation of RhpR is mediated by another TCS unit kinase RhpS, and helps in a sustained phosphorylated state. To achieve the phosphorylated RhpR abundance, another pathway is activated using its own promoter with an inverted repeat (IR) element, and their covalent bonding leads to producing more PRhpR. Unphosphorylated-RhpR has a positive regulatory effect on three genes a) functional accumulation of c-type cytochrome, b) regulate alcohol dehydrogenase synthesis, and c) positive modulation of *hemB* and negative effect on protease production in KB medium. RhpR also functions as a negative regulator of anthranilate synthase activity. On the other hand, phosphorylated RhpR inhibits swimming motility but shows a positive effect on the induction of twitching motility. Additionally, in vivo, P-RhpR functions as a negative regulator of the biofilm (*algD*) and c-di-GMP levels while improving lipopolysaccharide production in a positive manner. P-RhpR has a positive effect on LonB protein, which results in the breakdown of HrpR protein and prevention of HrpRS oligomerization. Gene orientations and scheme of events adapted with permission from Xie et al. (2019)

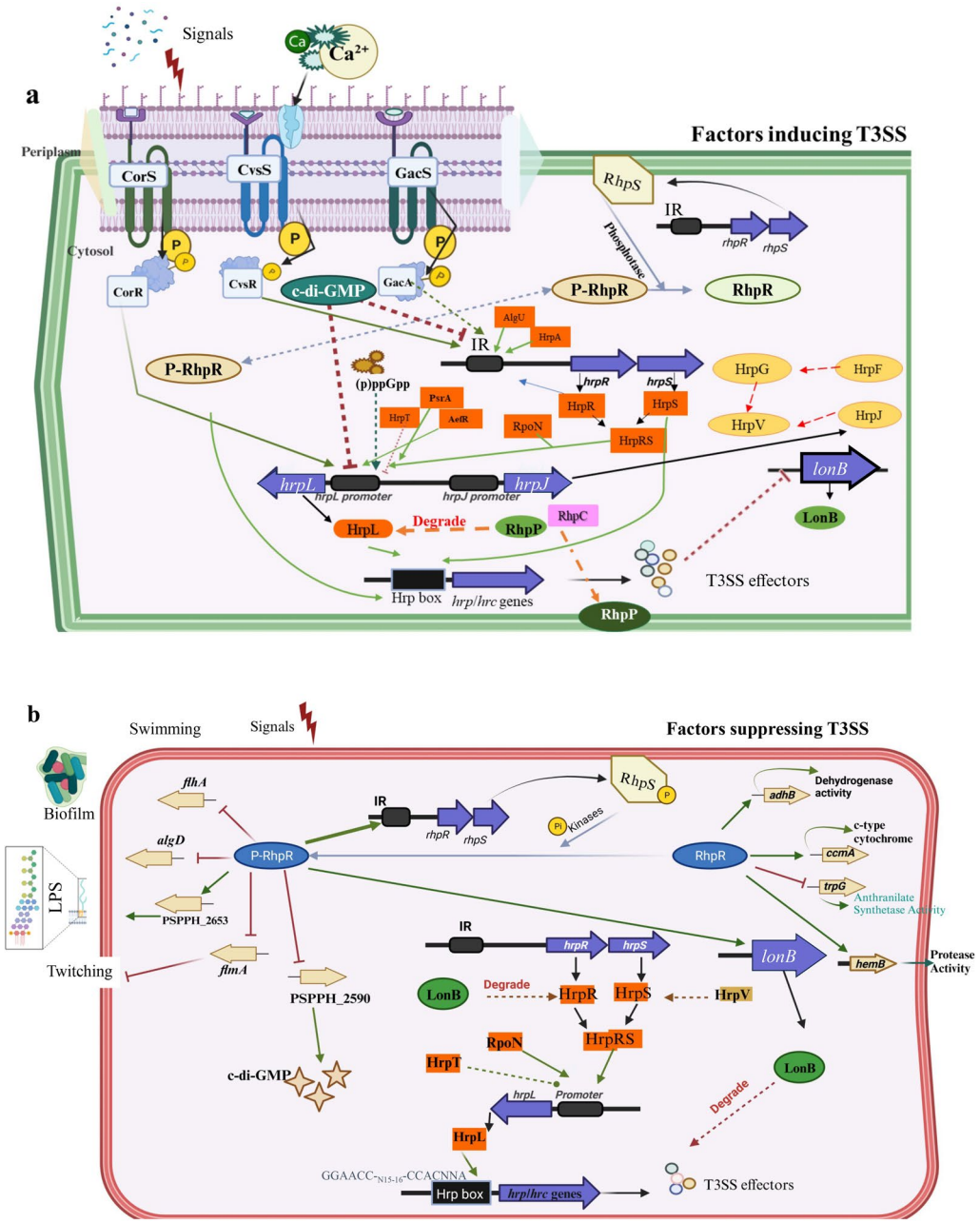



**Fig. 4** (See legend on previous page.)

to the cellular membrane by interacting with HrpG during stimulated T3SS expression. This spatial contact hinders the connection of HrpV with bacterial DNA and, therefore, inhibits the blockage effect of HrpS (Charova et al. 2018). The HrpVG complex still can interact with HrpJ in the inner membrane, but the HrpJ role within the cellular environment of plants is unidentified other than inhibiting the immune response of the host (Crabill et al. 2012). Before its translocation, HrpJ blocks the inner channel of the T3SS by creating a ternary complex resembling a gatekeeper, together with HrpVG (Cherradi 2013; Charova et al. 2018). In the inner core membrane of T3SS, another binding complex is formed by the interaction between HrpG and HrcU (Charova et al. 2018). During the infection of *P. syringae* inside the host plant, HrpJ acts as a facilitator for the complete functionality of the T3SS to secrete translocator proteins and harpins (Fu et al. 2006; Ramos et al. 2007; Crabill et al. 2012), depending on signals that induce secretion (Crabill et al. 2012).

Although the HrpA1 protein undergoes rapid degradation inside the bacterial cell, it can remain intact by forming a complex with the T3SS regulatory protein HrpF (Haapalainen et al. 2009; Huang et al. 2022). The importance of HrpF is well-established in disease pathogenesis and for the functional T3SS development in different *P. syringae* strains (Huang et al. 2016; Deng et al. 2017). This *hrpF* interaction may result in the de-repression of HrpS and the enhancement of the expression of the T3SS signaling cascade (Huang et al. 2016, 2022). In addition, elucidating the possible interactions of HrpF, HrpA1, and HrpJ-VG circuits could reveal their crucial role in intracellular dynamics and establish the regulatory link between the expression and secretion of the T3SS (Fig. 4). The HrpA1 protein is accumulated in the extracellular space before the secretion of other substrates of T3SS (Haapalainen et al. 2009; Waite et al. 2017). Overall, the pilus synthesis mediated by the *hrpA1* gene and subsequent interaction with host cells might potentially initiate the secretion process (Waite et al. 2017). However, the exact mechanisms behind the activation of downstream T3SS substrate secretion remain unknown.

The *hrp* genes in *Xanthomonas* spp. and *R. solanacearum* were classified with *hrp* group 2; they are different from *E. amylovora* and *P. syringae* in some aspects. *Xanthomonas* spp. and *R. solanacearum* use HrpG regulon to regulate it by HrpX, which interacts with a cis-element within the promoter region of *hrp* genes, referred to as plant-inducible promoter (PIP)-box, which is also present in the promoter of many T3 effectors ( Fan et al. 2017). In addition to HrpG and HrpX, HrpD6 was reported as a *hrp* regulator in *Xanthomonas* spp, responsible for regulating multiple *hrp* genes (Li et al. 2011; Fan et al. 2017). In addition to conserved genes, some extra regulatory and control genes, sigma factors are underway to discover.

### Activity of T3SS in artificial medium in relation to environmental cues

The T3SS-mediated virulence of Hrp1 species plays a crucial role, with strains investing substantial cellular resources in synthesizing the T3SS apparatus (Sturm et al. 2011). This allocation aims to quickly initiate the mechanism to counteract the host's immune system. Thus, the interaction between bacterial T3SS and the host's immunity is crucial in the initial stages of plant infection (Xin et al. 2018). Thus, the initial period during infection is critical in determining disease outcome and severity. To comprehend this critical phase of infection, especially in *P. syringae*, it is necessary to identify the specific signal from the pathogen that initiates T3SS activity. The *hrp*/*hrc* genes are generally repressed when the pathogen is grown in nutrient-rich environments. Nevertheless, a low level of expression may persist in these conditions due to the presence of genes such as *hrpV* and *hrpT* in T3SS cluster (O'Malley and Anderson 2021). The dissociation of the anti-repressor and anti-terminator complexes may occur under nutrient-deprived conditions at the *hrp*/*hrc* genes, facilitating a rapid increase in T3SS activity. However, additional evidence is required to support this hypothesis, providing opportunities for further investigation.

The expression of the T3SS-oriented *hrp*/*hrc* genes in *P. syringae* can be evaluated during growth in nutrient-deprived media (O'Malley and Anderson 2021). These nutrient-poor formulations, known as HIM (*hrp*-inducing minimal medium) and HDM (*hrp*-de-repressor medium), are designed to promote higher expression levels. The main difference lies in the carbon, nitrogen, and sugar sources (fructose or mannitol) and their slightly acidic pH. In addition, for *Xanthomonas* spp., *hrp*-inducing medium XOM2 is used to trigger T3SS activity. This environmental condition primes *P. syringae* for T3SS activation, highlighting the importance of understanding the nutritional cues that modulate T3SS expression in bacterial pathogenesis. It can be said that the expression of T3SS-associated genes in *P. syringae* and *Xanthomonas* may be influenced by broader environmental variables during infection, independent of specific plant metabolites acting as signals.

### Positive and negative regulation of T3SS levels by plant-derived signals

Similar to synthetic media conditions, studies have shown elevated expression levels of T3SS during infection in planta (Haapalainen et al. 2009), suggesting the existence of distinct signals in the rhizosphere microenvironment that trigger the production of the T3SS in bacteria. During initial investigations, it was observed that the series of T3SS genes in a cluster, especially *hrpL* expression was increased when two different host plants (susceptible and resistant) were infiltrated with *P. syringae* (Xiao et al. 2007). In addition to susceptible and resistant varieties, non-host species exposed to similar treatment revealed improved T3SS expression, suggesting metabolite-based signals are standard in different plants instead of being specific to the host plant. Previously, a study stated a significant ten-times rise in mRNA levels of *hrpA1* in a minimum medium supplemented with tomato exudates (soluble signals) (Haapalainen et al. 2009). Subsequently, the exudates obtained from *Arabidopsis*-based extracted suspension cultures without cells also induced the RNA levels of *hrpL* and levels of *avrPto* effector protein (Yan et al. 2019). In addition, the presence of plant cells in the suspension increased the proliferation of *P. syringae* DC3000 using the "infection-in-a-flask" approach. It

indicates that the signals originating from plants boosted bacterial proliferation by augmenting the deployment of the T3SS (Yan et al. 2019).

In another study, *Arabidopsis* seedlings immersed in water (exudates) resulted in significant *AvrPto* accumulation and enhanced *hrpL* gene expression level in *P. syringae* pv. *tomato* DC3000 (Anderson et al. 2014). Most importantly, citric acid and aspartic acid were identified as the bioactive molecules responsible for triggering the T3SS, as they are present in significant quantities in the tissues of plants. This finding further reinforces prior discoveries that T3SS is activated by common characteristics of the host signals instead of signals unique to a particular host (Rico and Preston 2008; Kumar et al. 2017; Yan et al. 2019). Although all bioactive substances retain a carboxyl group to exhibit acidity, not all can induce T3SS. For instance, amino acids such as leucine and valine may trigger T3SS, suggesting a certain degree of selectivity to detect these compounds. It also indicates that optimal biological activity mediated by acidic metabolites in triggering T3SS is dependent on the existence of an essential sugar like fructose. The generation of phytotoxins, such as syringomycin, in *P. syringae* pv. *syringae* is modulated by specific plant-derived signals, with the *syrB* gene playing a pivotal role in both syringomycin production and full virulence during plant pathogenesis. Research has demonstrated that phenolic glucosides, such as arbutin, phenyl-β-D-glucopyranoside, and salicin, act as strong inducers of *syrB* transcription, while sugars like D-fructose and sucrose enhance the pathogen's sensitivity to these phenolic signals, thereby amplifying toxin biosynthesis. These findings suggest that the synthesis of phytotoxins like syringomycin is closely linked to the pathogen's ability to detect and respond to plant metabolites, which may complement T3SS-mediated infection strategies during pathogenesis (Mo and Gross 1991).

In a quest to find a specific signal exuded from plants in regulating the effects of *P. syringae* infection, model organism *Arabidopsis* was mutated to impair the production of immunological regulator mitogen-activated protein kinases MAPK phosphatase 1 (MKP1) (Andrade et al. 2014). The *mkp1* mutant exhibited improved immune responses to pathogenic elicitors and increased resistance against *P. syringae* DC3000 infection. Moreover, in the context of *mkp1* infection, DC3000 exhibited a diminished capacity to stimulate T3SS-associated genes and transport effectors molecules (Anderson et al. 2011; 2014). Analysis of *mkp1* mutant exudates via GC–MS revealed decreased concentrations of several metabolites that induce T3SS, such as citric acid and aspartic acid. The complemented *MKP1* plant with these metabolites fully reestablished DC3000's capacity to transfer effectors, thereby regenerating susceptibility to DC3000 infection (Anderson et al. 2011; 2014). Overall, it can be inferred that the virulence-inducing signals depend on the plant host's genetic makeup during infection.

Auxin, also known as indole-3-acetic acid (IAA), is a plant-derived chemical capable of suppressing the production of *P. syringae*'s T3SS (Anderson et al. 2011; McClerklin et al. 2018; Djami-Tchatchou et al. 2020). As the infection progresses, *P. syringae* reallocates cellular resources from T3SS development to the other secretion system due to higher bacterial density in the apoplast, favoring microbial competition (McAtee et al. 2018; Djami-Tchatchou et al. 2020). Sulforaphane, a glucosinolate synthesized in *Arabidopsis* and other *Brassicaceae* family members, also suppresses T3SS gene expression by modifying the T3SS master regulator HrpS through covalent interactions, thereby enhancing plant defense (Wang et al. 2020). Meanwhile, the *Arabidopsis* plant lines unable to synthesize glucosinolate showed more susceptible action towards the infection of *P. syringae,* indicating that sulforaphane-mediated T3SS suppression contributes significantly to host defense mechanisms (Wang et al. 2020). Additional substances originating from plants, such as plant flavonoids and other phenolics (Vargas et al. 2013; Lee et al. 2015; Kang et al. 2020; Zhi et al. 2022), together with several synthetic chemicals, have shown inhibitory effects on the *P. syringae* T3SS (Ma et al. 2019; Puigvert et al. 2021). Considering the fact that the energy expenditure associated with the production and upkeep of the T3SS is substantial (Sturm et al. 2011), it is plausible that cells exhibiting low levels of T3SS expression, commonly referred to as "cheater" cells, can allocate their energy resources towards proliferation or other cellular activities. This mechanism may improve the pathogen population's adaptability and overall fitness (Xie et al. 2023). To fully grasp the impact of tactics on *P. syringae*'s virulence, it is crucial to comprehensively characterize the precise patterns responsible for initiating and sustaining T3SS bistability in the host environment.

Phenolic caffeic acid, secreted in increased amounts by tobacco roots following *R. solanacearum* inoculation, reduces disease symptoms when applied exogenously in pot and field experiments (Li et al. 2021). Other plant-derived natural products, such as coumarins, also exhibit antibacterial activity. For instance, 7-methoxycoumarin mitigated tobacco bacterial wilt in pot experiments (Han et al. 2021). Additionally, salicylic acid inhibits *R. solanacearum* growth and suppresses several bacterial wilt virulence factors (Lowe-Power et al. 2016). Plant signals as essential oils from aromatic plants contain various volatile molecules with protective effects against many phytopathogens, including *R. solanacearum* (Pradhanang et al. 2003; Deberdt et al. 2018). These oils have been

studied for over 20 years for their efficacy against this bacterium (Raveau et al. 2020).

In *Ralstonia* and *Xanthomonas*, the enzymatic functions of some type III effectors (T3Es) include acetyltransferases, proteases, and ubiquitin ligases, with established repertoires of plant proteome interactors (González-Fuente et al. 2020). T3Es can have multiple functions depending on the host; for example, RipAB downregulates calcium signaling in potatoes (Zheng et al. 2019) and inhibits TGA transcription factors in *Arabidopsis* (Qi et al. 2022). Furthermore, screening of various plant-based chemicals against these strains resulted in the identification of salicylidene acylhydrazides as T3SS inhibitors, which limits bacterial growth in plants (Puigvert et al. 2021).

Host-derived amino acids and sugars, especially in the apoplast which serves as a battleground where pathogens attempt to breach the plant's defenses and establish infection in leaves (Rico and Preston 2008; Anderson et al. 2014), are the first compounds encountered by *P. syringae* throughout the process of infection. These plant exudates contain organic acids and amino acids that optimally trigger T3SS genes after their inclusion in the induction medium (minimal medium). In order to understand the regulatory T3SS pathway in response to these organic acids and amino acids, a notable gene was identified by the genetic mutations known as SetA that induce the T3SS genes in response to sugars, and is a putative transcription factor of the DeoR-type (Turner et al. 2020). The gene SetA alone has been implicated in partially increasing the expression levels of the effector genes *avrRpm1* and *hrpL* in DC3000 upon exposure to sucrose, fructose, and mannitol. In *Arabidopsis* leaves, it was identified as an essential factor for achieving the highest *hrpL* expression (Turner et al. 2020). A mutant strain of DC3000 lacking the *setA* gene exhibited reduced growth capability, accompanied by impaired expression of *hrpL*. Furthermore, the observed activity of *hrpRS* and *rpoN* was quite like the wild type, suggesting a role for SetA in directly regulating *hrpL* induction or post-translational feedback modulation of unaffected genes (Turner et al. 2020).

### T3SS regulatory pathway in response to environmental stimuli

The regulation of T3SS by host signals is intricate, involving multiple sensory and signaling pathways in combination that detect the signals from the host and respond to environmental stimuli, ultimately impacting T3SS deployment (Ma et al. 2019). According to recent advances, HrpL and its associated co-regulating components, HrpRS, are used to sense the environmental cues in *P. syringae*, resulting in a canonical cascade (Khokhani et al. 2013). Its network tightly regulates the T3SS due to its shared nature among Gram-negative pathogens. However, different pathogens of various types use unique master regulators of T3SS. For example, comparatively, Hrp1-type pathogens initiate the T3SS pathway from HrpS, while in Hrp2-type, T3SS is initiated by HrpG (Fig. 4b). Further research has expanded our knowledge of the Hrp1 and Hrp2 regulatory networks, revealing their modulation by key regulatory pathways such as the Gac-Rsm pathway, the C-di-GMP second messenger pathway, and quorum sensing (Fig. 4b). For instance, the global regulatory factor ExsA is influenced by factors like cAMP, Gac-Rsm, and RpoS (sigma factor), independent of T3SS, exerting direct or indirect regulatory effects on T3SS transcription and function (Fig. 4; Anantharajah et al. 2016). However, our understanding of the sensory domains and the environmental stimulus inducing T3SS in *P. syringae* is limited.

The involvement of the two-component systems (TCSs) in regulating bacterial virulence in the environment is a regular occurrence (Beier and Gross 2006; Tang et al. 2006). Bacteria predominantly use TCSs for signaling and communication, which consist of a membrane-integrated histidine kinase that perceives a stimulus and a cytoplasmic response regulator. The exchange of information by histidine kinase (sensory unit) and a response regulator through conserved phosphorylation and dephosphorylation reactions (Jung et al. 2012; Sankhe et al. 2023). After the perception of stimulus by the sensory first component, it is transferred to the cognate transcriptional factor and response regulator to regulate gene expression (West and Stock 2001). A couple of years ago, the two-component system AauSR was discovered by the Tn5-mediated genetic screening of DC3000. This research further revealed that the identified TCS could utilize amino acids, thus designating it as an Amino-Acid Utilization Sensor and Response Regulator (AauSR). This nutrient-sensing system also comprises a histidine kinase (AauS) and response regulator AauR and TCS was primarily responsive to aspartic acid and fructose, resulting in augmented expression of T3SS genes (Yan et al. 2020). These two genes were located inside the *aat*/*aau* locus, widely conserved in *Pseudomonas* strains. This locus is also comprised of genes encoding the ABC transporter complex AatQMP linked to another protein, AatJ, present in the periplasm. Previously, this locus's roots were identified and described in nonpathogenic *P. putida* (Sonawane et al. 2006; Singh and Röhm 2008). Aspartic acid and glutamic acid consumption in *P. putida* is facilitated by the proteins AatQMP and AatJ. Additionally, the protein AauS is responsible for sensing and transporting the substrates that have accumulated in cells. The *aatJ* gene was supposed to have a binding site regarded

as AauR binding motif (Rbm). Upon exposure to aspartic acid or glutamic acid, a series of events used to happen in a sequence that includes AauR finds its binding site in the *aatJ* gene followed by activation of ABC co-transporter locus *aatJQMP*, which leads to improvement and enhanced *hrpRS* and *hrpL* gene expression (Deng et al. 2014; Yan et al. 2020). The genome mining of DC3000 for Rbm sequences revealed an extra Rbm sequence located upstream of *hrpRS*, suggesting a potential direct influence of AauR on the production of *hrpRS* (Fig. 4).

Deletion of the Rbm upstream of *hrpRS* exhibited reduced T3SS gene activity levels in response to aspartic acid and glutamic acid, suppressing virulence of DC3000 in *Arabidopsis* plants (Deng et al. 2014; Yan et al. 2019). The presence of Rbm in the *hrpRS* promoter is conserved among 38 *P. syringae* isolates with the canonical tripartite pathogenicity island, suggesting a common regulatory function of AauSR towards HrpRS (Fig. 4). A similar universal nature of the motif of each locus was observed in *P. syringae* B728a, where *aauR* essentially increased T3SS gene activity, resulting in heightened strain virulence in bean plants (Vinatzer et al. 2006; Kumar et al. 2017; Yang et al. 2017). All these events proved that the Rbm incorporation happened earlier than *hrpRS* motif in *P. syringae* during evolution, likely playing a key role in the initial development of *P. syringae* virulence (Kumar et al. 2017).

#### Redundancy in different global regulatory pathways on T3SS regulation

Three different TCS localized in the periplasmic space to the inner membrane are identified and widely described in *P. syringae* strains. One of the two-component systems, GacSA, is responsible for responding to an unidentified stimulus and subsequently activating the cytoplasmic response regulator GacA through a process known as phosphorelay (Latour 2020). Earlier studies on TCS in *P. syringae* initially designated the *gacS* gene as *lemA* (*lesion manifestation*). However, It was a difficult task for authors to pinpoint the associated response regulator *gacA* essentially present in the vicinity of the response regulator, despite the common occurrence of two units being co-located in the genome (Koretke et al. 2000). This system bears a resemblance to the BarA/UvrY TCS found in *E. coli* (Pernestig et al. 2001). In the γ-proteobacteria, the *gacA* is located distant from *gacS* and co-located with *uvrC* in an operon. The *uvrC* gene encodes a component of the nucleotide excision repair complex (Heeb and Haas 2001; Heeb et al. 2002).

Certain studies described a positive effect of GacSA on T3SS expression (Chatterjee et al. 2003, 2007; Vargas et al. 2013; Ferreiro et al. 2018), while others have documented a negative regulation by GacSA and suggested that it is not essential to initiate T3SS expression in planta conditions (Marutani et al. 2007). For instance, GacSA suppresses the T3SS in *P. aeruginosa* (Brencic et al. 2009; Valentini et al. 2018). In another study focused on investigating the molecular mechanisms behind the regulation of virulence by GacSA in *P. syringae* (O'Malley et al. 2019), an AC811 mutant strain exhibited reduced virulence and a decreased T3SS activity in the plant microenvironment. This model has been represented in several contemporary publications (Brencic and Winans 2005; Tang et al. 2006; Moll et al. 2010), as well as in different predictive biological models of virulence regulation in *P. syringae* (MacLean and Studholme 2010).

In addition, the deactivation of *gacA* revealed increased expression in an AC811 strain rather than a reduction of T3SS activity (O'Malley et al. 2019). The diminished virulence of an AC811 mutant strain was ascribed to a secondary alteration in *anmK*, encoding an enzyme implicated in the reprocessing of the bacterial cell wall. It showed adverse effects on *uvrC`s* transcriptional levels, contributing to the observed attenuation. However, the association of *P. syringae* virulence with *anmK* and *uvrC* has not been previously established. Thus, a *gacA* mutation can polarize the downstream expression of *uvrC*, suggesting a similar adverse impact on the virulence of *P. aeruginosa* infections in mammals (Ferreiro et al. 2018; Qin et al. 2022).

Moreover, in contrast to the deployment of T3SS, the presence of GacA was necessary for the cell motility of DC3000, suggesting an inverse regulatory relationship between the T3SS and motility (O'Malley et al. 2019). Frequent cell movement (motility) is essential for *P. syringae* attachment with the host to enter the intercellular space via stomatal openings in the surface of leaves (Chieda et al. 2005). Consequently, this process may enhance the ability of *P. syringae* to survive on the surface of plants until enter the internal tissues of leaves. The GacSA system is subsequently inactivated, leading to the de-repression of T3SS at the apoplast (Fig. 4a). The precise mechanism(s) underlying the impact of GacSA on the deployment of T3SS remains uncertain, but it is established that GacSA exerts regulatory control over the RsmA proteins.

Another TCS, RhpSR, is responsible for regulating the expression of T3SS in *P. syringae* pv. *phaseolicola* 1448a. The response regulator RhpR undergoes phosphorylation to exert repression on T3SS through its interaction with an IR element located in the promoters of *hrpR* and/or *hopR1* (Xiao et al. 2007; Deng et al. 2010). Furthermore, the phosphorylated form of RhpR can activate the promoter activity of the Lon protease gene, leading to the degradation of HrpR (Xiao et al. 2007; Deng et al. 2010; Xie et al. 2019). The sensor histidine kinase, RhpS, can show autokinase activity, including kinase and

phosphatase activity towards RhpR (Deng et al. 2010; 2014). During the abundance of nutrients in a medium, the protein RhpS acts as a kinase to facilitate the suppression of T3SS. In contrast, RhpS exhibits phosphatase activity towards RhpR for the induction of T3SS in minimal nutrition. This activity leads to a decrease in the activity of RhpR at specific target promoters (Xiao et al. 2007; Xin et al. 2016, 2018; Xie et al. 2019). RhpR can undergo phosphorylation in addition to RhpS, perhaps by the action of physiological phosphor-donors like acetyl phosphate, and/or by sensor kinases from other TCSs (Deng et al. 2017). This regulatory process is achieved through a negative feedback loop (Deng et al. 2010; Xin et al. 2018). The RhpSR system may play a role in the co-regulation of T3SS and cellular maintenance, potentially enabling a broader interchange of metabolism and pathogen virulence in response to environmental factors, such as variations in nutrition accessibility (Zhou et al. 2015; 2016; Xie et al. 2019). Nevertheless, the precise environmental stimuli that regulate the activity of RhpSR have yet to be determined.

Another well-documented regulatory pathway, the calcium-induced TCS CvsSR can influence the T3SS genes (Fishman and Filiatrault 2019). The CvsSR expression was significantly accumulated in DC3000 when exposed to exudes derived from the leaves of tomatoes and by a calcium ($Ca^{2+}$) cation (Fishman et al. 2018). The plant apoplast contains plenty of $Ca^{2+}$ ions, and it goes on increasing during bacterial infection in plants (Stael et al. 2011). Moreover, the phosphorylated response regulator CvsR binds to the inverted repeat element of *hrpR* and *hrpS* and some other regions inside the T3SS effector genes in the presence of calcium supplementation in T3SS-inducing minimal media and activates T3SS expression. The strain impaired in *cvsS*/*cvsR* resulted in reduced proliferation and disease symptoms on host plants, suggesting that CvsSR has a direct role in the virulence of *Pseudomonas* strains (Fishman et al. 2018; Fishman and Filiatrault 2019). The CvsSR system has been found to indirectly inhibit the expression of AlgU, which was responsible for the positive regulation of the T3SS. The specific method by which CvsSR affects *algU* expression remains unknown, suggesting that the impact of CvsSR on T3SS dynamics may be intricate and multifaceted (Stael et al. 2011).

## Inhibitors of the T3SS genes in pathogenic bacteria

T3SS inhibitors primarily consist of small molecular compounds that specifically interfere with the structural synthesis or functional expression of T3SS (Grier et al. 2010). Unlike traditional antibiotics, which target pathogen growth, T3SS inhibitors act specifically on the T3SS, significantly reducing the selective pressure driving pathogen resistance (Slepenkin et al. 2011). Through extensive research on T3SS structure, expression regulation, and virulence (Asif et al. 2024a), a variety of plant extracts and small molecule T3SS inhibitors are reported (Yang et al. 2008; Puigvert et al. 2021; Wang et al. 2020). Screening T3SS inhibitors on plant pathogens has yielded analogs of some inhibitors found in animal pathogens and novel plant-derived and chemically synthesized compounds (Slepenkin et al. 2011). Initial screening efforts were focused on animal pathogens such as *P. aeruginosa* and *Yersinia* species, and their efficacy was proven for other pathogenic strains (Kowal 2013; Aguilera-Herce et al. 2023).

In the search for effective inhibitors targeting the T3SS in plant pathogens, several promising compounds have been identified. Salicylidene acylhydrazides, for instance, inhibit T3SS gene expression in *R. solanacearum*, reducing its virulence (Puigvert et al. 2021). Phenolic compounds like caffeic acid, released by plant roots in response to pathogen attacks, have also shown efficacy in reducing disease symptoms by affecting T3SS regulation in *R. solanacearum* (Li et al. 2021). Coumarins, such as 7-methoxycoumarin, mitigate tobacco bacterial wilt caused by *R. solanacearum* through their impact on T3SS (Han et al. 2021). Additionally, salicylic acid has been shown to inhibit T3SS in *R. solanacearum* by repressing several virulence factors (Lowe-Power et al. 2016). Cyclohexanone derivatives interfere with T3SS in *X. campestris*, reducing pathogenicity, while quinazolinone derivatives prevent T3SS assembly in *Xanthomonas* species (Yang et al. 2017). Moreover, 2-imino-5-arylidene thiazolidinone inhibits T3SS in *E. amylovora*, a pathogen causing fire blight in apple and pear trees, by blocking the secretion of T3SS effectors (Anantharajah et al. 2017). These inhibitors offer promising leads for developing new control strategies against bacterial plant diseases by targeting T3SS, a critical virulence factor in many plant pathogens.

Further research is required to elucidate the mechanism of action of these compounds and explore their potential value for preventing and controlling animal and plant diseases. Different inhibitors have different targets (Table 1), but we focused on three categories: inhibiting T3SS regulatory factors genes, inhibiting T3SS structural genes, and inhibiting T3SS effector proteins.

### *T3SS regulatory factor inhibitors*

Plants can produce various secondary metabolites to resist pathogen infection. Most of these metabolites are toxic to pathogenic bacteria (Wang et al. 2020). Sulforaphane (SFN) crude extracts was tested against DC3000 which damaged the expression of effector protein AvrPto, ultimately inhibits its T3SS expression

**Table 1** Reported chemicals involved in inhibiting type III secretion systems

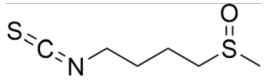
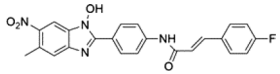
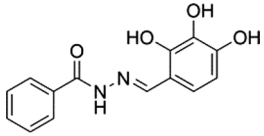
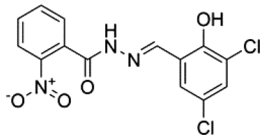
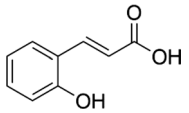
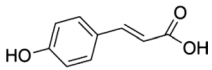
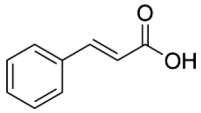
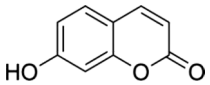
| Chemical name/Structure | Pathogen | Mechanism | References |
|---|---|---|---|
|  Sulforaphane | *P. syringae* | Cys 209 of HrpS, which regulates T3SS expression | Wang et al. (2020), He et al. (2023) |
|  *N*-hydroxybenzimidazoles | *P. aeruginosa; Y. pseudotuberculosis* | Combined with the C- terminal of ExsA prevent binding of ExsA to the promoter region | Grier et al. (2010), Marsden et al. (2016) |
|  Benzoic acid N′-(2,3,4-trihydroxybenzylidene)-hydrazide | *E. amylovora; R. solanacearum* | Inhibition of multiple T3SS regulatory genes; Inhibition of HrpB expression in both strains | Puigvert et al. (2021), Yang et al. (2017) |
|  2-nitro-benzoic acid N′-3,5-dichloro-2-hydroxy. benzylidene)-hydrazide | *R. solanacearum* | Directly inhibit T3SS expression by inhibiting HrpB | Puigvert et al. (2021) |
|  O-coumaric acid | *E. amylovora; X. oryzae* pv. *oryzae; D. dadantii* | It inhibits HrpX regulatory pathway of T3SS expression; Target RsmA/RsmB pathway in *D. dadatii* by improving HrpL expression | Fan et al. (2019), Fan et al. (2017), Li et al. (2015), Yang et al. (2008) |
|  P-coumaric acid | *D. dadantii; R. solanacearum* | Inhibition of the HrpX-HrpY regulatory pathway | Li et al. (2015), Lv et al. (2021) |
|  T-cinnamic acid | *E. amylovora; D. dadantii* | Inhibition of T3SS expression in *D. dadatii*, but promote T3SS expression in *E. amylovora* | Fan et al. (2019), Yang et al. (2008) |
|  Umbelliferone | *R. solanacearum* | Inhibiting T3SS expression by directly inhibiting HrpG expression | Yang et al. (2017),Yang et al. (2016) |

**Table 1** (continued)

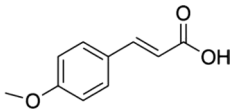
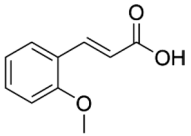
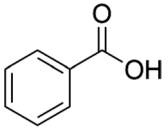
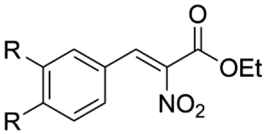
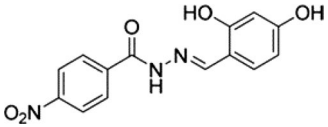
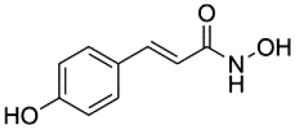
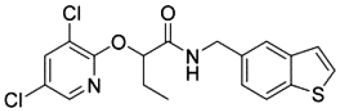
| Chemical name/Structure | Pathogen | Mechanism | References |
|---|---|---|---|
|  4-methoxy.cinnamic acid | *E. amylovora* | Inhibition of T3SS expression by inhibiting the HrpS-HrpL pathway | Khokhani et al. (2013) |
|  Trans-2-methoxycinnamic acid | *X. oryzae* pv. *oryzae* | Inhibition of T3SS expression through the HrpG/HrpX regulatory pathway | Fan et al. (2017) |
|  Benzoic acid | *E. amylovora* | Inhibition of T3SS expression by inhibiting the HrpS/HrpL pathway | Khokhani et al. (2013) |
|  Ethyl 2-nitro-3-arylacrylates | *X. oryzae* pv. *oryzae* | Inhibition of T3SS expression through the HrpG/HrpX regulatory pathway | Jiang et al. (2019), Shi et al. (2023) |
|  Salicylidene acylhydrazides | *P. aeruginosa; Y. pseudotuberculosis; C. trachomatis;* EHEC; *E. amylovora; R. solanacearum; S. typhimurium*; *Shigella* spp. | Directly affects the action of three enzymes: WrbA, Tpx, and FolX in *P. aeruginosa*, EHEC, *C. trachomatis*, and influences the assembly of T3SS needle-like structures in *Shigella* spp; Inhibiting the formation of T3SS base protein in *S. Typhimurium* | Anantharajah et al. (2017), Gill et al. (2015), Negrea et al. (2007), Slepenkin et al. (2011), Tree et al. (2009) |
|  Trans-4-hydroxycinnamodydro xamic acid | *P. aeruginosa; E. amylovora; D. dadantii* | Inhibition of GacS/GacA in *P. aeruginosa*, RsmYZ-RsmA-ExsA regulatory pathway, and RsmB in *D. dadatii* and *E. amylovora*<br>Influences RsmA-HrpL regulatory pathway | Khokhani et al. (2013), Li et al. (2015) |
|  Phenoxyacetamide | *P. aeruginosa; Y. pseudotuberculosis; C. trachomatis;* | Interacts with the needle-like structure and disrupts its function | Aiello et al. (2010), Anderson (2023) |

**Table 1** (continued)

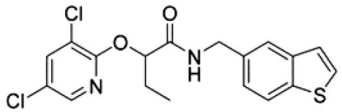
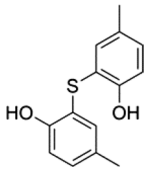
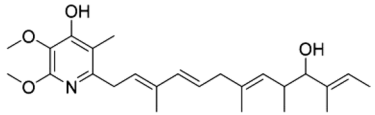
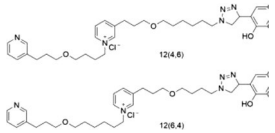
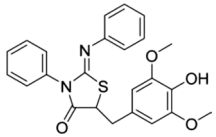
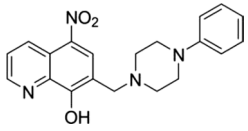
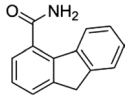
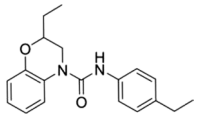
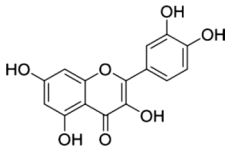
| Chemical name/Structure | Pathogen | Mechanism | References |
|---|---|---|---|
|  Resveratrol oligomers | *P. syringae* | Inhibit the expression of HrpL and HrpA, and disrupt pili structure | Kang et al. (2020) |
|  2,2-thiobis-(4-methylphenol) | *P. aeruginosa; Y. pseudotuberculosis* | Inhibits the chaperone protein LcrH, *cmpD*, resulting in the loss of T3SS structure | Jessen et al. (2014) |
|  Piericidin A | *P. aeruginosa; Y. pseudotuberculosis* | Inhibiting the synthesis of T3SS needle-like structure | Duncan et al. (2014) |
|  Clioquinol derivatives) 3-alkylpyridine pyridinium alkaloid | *P. aeruginosa* | Suppress the secretion of T3SS effector ExoT and ExoS | Ngo et al. (2019) |
|  Thiazolidinones | *P. aeruginosa; Y. enterocolitica; S. enterica* | Inhibition of T3SS base protein synthesis, repression of SipA and SspH1 flagella motility | Kline et al. (2009), Tao et al. (2019) |
|  Hydroxyquinolines | *P. aeruginosa; Y. pseudotuberculosis; C. trachomatis* | Inhibition of ATPase and flagellar synthesis of T3SS | Anantharajah et al. (2017) |

**Table 1** (continued)

| Chemical name/Structure | Pathogen | Mechanism | References |
|---|---|---|---|
|  Pseudolipasin A | *P. aeruginosa* | Inhibition of T3SS effector ExoU activity | Lee et al. (2007) |
|  Exosin | *P. aeruginosa* | Inhibition of T3SS effector ExoS activity | Arnoldo et al. (2008), Lee et al. (2007) |
|  Quercetin | EHEC | Inhibiting the adhesion ability of T3SS effectors | Xue et al. (2019); Xue and Zhu (2023) |

(Table 1; Wang et al. 2020). Further analysis revealed that SFN directly acted on the 209 amino acids of HrpS in DC3000, and competitively bind to HrpS to suppress the expression of T3SS. N-dihydroxybenzenes were obtained by screening the interaction between *E. coli* AraC family regulatory factors (such as ExsA) and DNA binding regions. N-dihydroxybenzene binds to the C-terminal of *P. aeruginosa* ExsA, thereby preventing the binding of ExsA to the DNA promoter region. The biological activities of N-dihydroxybenzenes (Table 1) include reducing T3SS gene expression and T3SS-mediated in vitro virulence (Grier et al. 2010; Marsden et al. 2016).

#### *T3SS regulatory pathway inhibitors*

Most of the T3SS inhibitors are hydroxycinnamic acid and its derivatives. Using the *hrpA* promoter reporter vector (pHrpA*)* and GFP-gene, hydroxycinnamic acid compounds from plant metabolites were screened by flow cytometry. Among them, *o*-coumaric acid and *p*-coumaric acid reduce the expression of T3SS by inhibiting related genes in the HrpX-HrpY pathway in *E. amylovora* (Li et al. 2015; Fan et al. 2017), while 4-methyl cinnamic acid and trans-2-methyl cinnamic acid (Table 1) subdued HrpS-HrpL pathway in *X. oryzae* pv. *oryzae* and *E. amylovora*, respectively. They can also inhibit T3SS expression related to the HrpG linked to HrpX pathway in rice pathogen (Fan et al. 2017, 2019; Shi et al. 2023). Benzoic acid and ethyl-2-nitro-3-acrylates affect the HrpS-HrpL pathway in *the Xanthomonas* strain (Khokhani et al. 2013; Jiang et al. 2019).

#### *Transcriptional and post-transcriptional pathway inhibitors affecting T3SS*

Salicylidene acetylhydrazides (SA) compounds are the class of T3SS inhibitors widely reported, explored, and first identified against the pathogenic bacterium *Y. pseudotuberculosis*. It can help mice resist the infection of *Y. pseudotuberculosis* and EHEC. SA compounds directly inhibit the activity of three secondary metabolic synthase enzymes, WrbA, Tpx, and FolX, which indirectly affect the transcription of flagellar synthesis genes and T3SS regulatory genes, thereby exerting the function of inhibiting T3SS (Slepenkin et al. 2011; Anantharajah et al. 2017). Trans-4-hydroxycinnamic acid (TMCA), screened from plant phenols, inhibits GacSA, RsmYZ-RsmA, and ExsA mediated regulatory pathway (Table 1). These compounds induce and regulate the expression of small RNA genes *rsmY* and *rsmZ*, which compete for RsmA to inhibit the expression of ExsA in *P. aeruginosa*. Subsequent studies have found that TMCA can also inhibit plant pathogens *D. dadantii*, and the RsmB-RsmA-HrpL regulatory pathway in *E. amylovora* extends the inhibitory range of TMCA from animal pathogens to plant pathogens (Khokhani et al. 2013).

#### *The needle/pilus structure and base protein formation inhibitors*

The needle structure of T3SS is crucial for its functional performance, and the scale protein SctP can detect needle length during the synthesis process of needle-like structures. In plant pathogens, poorly developed needle-like structures can affect the pathogen's contact with the host's cytoplasm, thereby affecting the transport of effectors. The most direct ways to suppress needle-like structures include subunit polymerization, inducing incorrect subunit folding, physically hindering the binding between subunits, or altering subunit polymerization. SA compounds also show inhibitory effects on the T3SS of *S. typhimurium*, *Shigella* spp., EHEC, *X. oryzae*, and *E. amylovora* (Anantharajah et al. 2017; Fan et al. 2017; Tao et al. 2019). SA did not affect the expression level of needle-like structure synthesis subunits in T3SS, thus suggesting that the assembly of T3SS was affected. Subsequent observations under electron microscopy indicated that the number of T3SS injection devices per cell decreased by 30.40% after SA treatment to *Shigella* spp., and the number of poorly developed needle-like structures significantly increased. Therefore, SA is considered a class of inhibitors that affect the assembly of needle-like structures (Tao et al. 2019). The T3SS base attaches needle-like structures to bacterial cell membranes with two main parts: a lower ring connected to the inner membrane and an upper ring connected to the outer membrane through the periplasm. Due to the conserved nature of these base proteins across secretion systems and flagellar structures, targeting T3SS base proteins for inhibitors could lead to unintended effects (Abby and Rocha 2012; Diepold et al. 2015). Thiazolidinones, particularly 2-imino-5-arylidene thiazolidinones (Table 1), interact with SctC, inhibiting base proteins in *S. typhimurium* T3SS and reducing effector secretion (Felise et al. 2008). This inhibition is achieved by targeting the HBF1 homologous protein of SctC, affecting the effectors' excretion, like SipA and SspH1, without impacting flagella motility (Kline et al. 2009). Considering their pedestal-like structure and conservation, HBF1 family proteins are promising targets for broad-spectrum T3SS inhibitors. The compound MBX2359, identified using a *luxCDABE* reporter system and the ExoT effector gene, and phenoxyacetamides discovered through screening over 80,000 compounds exemplify promising avenues for T3SS inhibitor development (Aiello et al. 2010).

### Future prospects

Over the last 25 years since its discovery, significant studies have been undertaken to unravel the intricacies of the T3SS nanomachine. Despite the advancements, challenges and gaps in knowledge that exist, future research endeavours should prioritize unravelling the molecular mechanisms controlling the priority of secretion processes within a biological system, understanding the perception of secreted signals, and establishing a unified model for exploring the functions of effectors in relation to pathogenesis. Small genetic changes can fundamentally alter T3SS control, leading to diverse variations adapted to the specific lifestyles of different pathogens. Current research advancements highlight the incomplete understanding of T3SS regulation, emphasizing the need to explore molecular mechanisms, including regulatory RNAs, under different infection conditions. Additional fundamental questions left unanswered include: Is the concept of an effector network universally applicable among pathogens utilizing a T3SS? Future research directions in understanding bacterial pathogenesis could focus on elucidating the essential cellular processes targeted for sustained infection and identifying potential targets within these pathways. Investigating how molecular mechanisms identified in one bacterial strain extend to other strains, related species, or across bacterial families would provide insights into common virulence strategies and potential vulnerabilities. The study of post-transcriptional control elements, such as sensory and regulatory RNAs, and their adaptation to intrinsic changes and regulatory rewiring is particularly intriguing. Understanding how these elements contribute to strain-specific variations advantageous for bacteria in diverse host niches could unveil novel targets for intervention and offer strategies for combating bacterial infections effectively.

Certain other basic trending questions include: Can artificial intelligence be implied to predict the essential cellular processes regarding T3SS? What are the molecular targets of current T3SS inhibitors, and how do they interact with these targets? Can inhibitors' specificity, potency, and efficacy be enhanced by leveraging our understanding of T3SS function? How quickly will resistance to T3SS inhibitors emerge, and can it be managed through dose optimization, combination therapy, or the development of multi-target molecules similar to antibiotic strategies? What are the cellular repercussions when T3SS is inhibited? Does inhibition impose fitness costs, disrupt other virulence factors, trigger stress responses, interfere with quorum sensing, or induce resistance mechanisms?

## Conclusions

The T3SS protein transports nanomachines found in most Gram-negative bacterial pathogens, which transport effector proteins into the host cell cytoplasm, spanning the host cell membrane and causing infection. Many plant pathogens, like *P. syringae*, *Ralstonia* spp., and *Xanthomonas* spp., possess these T3SS apparatuses, and their associated diseases are the cause of severe economic losses. A wide diversity of the hosts is affected by the T3SS-possessing pathogen, and their T3SS apparatus and secretion mechanisms are remarkably conserved. The probable interaction of the pathogens with the host and environmental cues, particularly regulate the response of phytobacterial T3SS. Different studies found conflicting results regarding GacSA's effect on T3SS expression, especially in *P. syringae*. Certain studies described it as a positive regulator, while others indicated it as a negative regulator. TCS-oriented complications can be resolved by species and strain-specific studies. In addition to pesticides and antibiotics, T3SS inhibitors precisely target the T3SS instead of disturbing pathogen growth and cause no risk for resistance development. Different inhibitors have different targets, especially inhibiting regulatory factors genes, damaging functionality of structural genes forming proteins, and suppressing effector protein secretion. These target sites could be useful to develop more novel drugs to inhibit T3SS.

#### Abbreviations

| | |
|---|---|
| AauSR | Amino-acid utilization sensor and response regulator |
| CvsSR | Calcium-induced two-component system |
| EHEC | Enterohemorrhagic *Escherichia coli* |
| EPEC | Enteropathogenic *Escherichia coli* |
| IAA | Indole-3-acetic acid |
| LemA | Lesion manifestation A |
| RBM | AauR-binding motif |
| SA | Salicylidene acetylhydrazides |
| SctP | Scale protein |
| SFN | Sulforaphane |
| T3SS | Type III secretion systems |
| TCS | Two component system |
| TMCA | Trans-4-hydroxycinnamic acid |

#### Acknowledgements

Special thanks to the China Postdoctoral Science Foundation (353881).

#### Author contributions

MA conceptualized and prepared the project outline, wrote the original draft, and generated the illustrations; XX and ZZ reviewed, improved, and edited. All the authors approved the final version.

#### Funding

The National Natural Science Foundation of China (Grant No. 32001864, 32460655), and the earmarked fund for the China Agriculture Research System (Grant No. CARS-26-04A).

#### Availability of data and materials

Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no conflict of interest.

Received: 8 May 2024 Accepted: 20 December 2024
Published online: 20 February 2025

**References**

Abby SS, Rocha EPC. The non-flagellar type III secretion system evolved from the bacterial flagellum and diversified into host-cell adapted systems. PLoS Genet. 2012;8:e1002983. https://doi.org/10.1371/journal.pgen.1002983.

Abrusci P, Vergara-Irigaray M, Johnson S, Beeby MD, Hendrixson DR, Roversi P, et al. Architecture of the major component of the type III secretion system export apparatus. Nat Struct Amp Mol Biol. 2013;20(1):99–104. https://doi.org/10.1038/nsmb.2452.

Aguilera-Herce J, Panadero-Medianero C, Sánchez-Romero MA, Balbontín R, Bernal-Bayard J, Ramos-Morales F. *Salmonella* type III secretion effector SrfJ: a glucosylceramidase affecting the lipidome and the transcriptome of mammalian host cells. Int J Mol Sci. 2023;24(9):8403. https://doi.org/10.3390/ijms24098403.

Aiello D, Williams JD, Majgier-Baranowska H, Patel I, Peet NP, Huang J, et al. Discovery and characterization of inhibitors of *Pseudomonas aeruginosa* type III secretion. Antimicrob Agents Chemother. 2010;54:1988–99. https://doi.org/10.1128/aac.01598-09.

Alfano JR, Charkowski AO, Deng W-L, Badel JL, Petnicki-Ocwieja T, van Dijk K, et al. The *Pseudomonas syringae* Hrp pathogenicity island has a tripartite mosaic structure composed of a cluster of type III secretion genes bounded by exchangeable effector and conserved effector loci that contribute to parasitic fitness and pathogenicity in plants. Proc Nat Acad Sci. 2000;97:4856–61. https://doi.org/10.1073/pnas.97.9.4856.

Alvarez-Martinez CE, Sgro GG, Araujo GG, Paiva MRN, Matsuyama BY, Guzzo CR, Andrade MO, Farah CS. Secrete or perish: the role of secretion systems in *Xanthomonas* biology. Comput Struct Biotechnol J. 2020;19:279–302. https://doi.org/10.1016/j.csbj.2020.12.020.

Anantharajah A, Mingeot-Leclercq M-P, Van Bambeke F. Targeting the type three secretion system in *Pseudomonas aeruginosa*. Trends Pharmacol Sci. 2016;37(9):734–49. https://doi.org/10.1016/j.tips.2016.05.011.

Anantharajah A, Buyck JM, Sundin C, Tulkens PM, Mingeot-Leclercq M-P, Van Bambeke F. Salicylidene acylhydrazides and hydroxyquinolines act as inhibitors of type three secretion systems in *Pseudomonas aeruginosa* by distinct mechanisms. Antimicrob Agents Chemother. 2017;61(6):e02566-e2616. https://doi.org/10.1128/AAC.02566-16.

Anderson JC. Ill communication: host metabolites as virulence-regulating signals for plant-pathogenic bacteria. Ann Rev Phytopathol. 2023;61:49–71. https://doi.org/10.1146/annurev-phyto-021621-114026.

Anderson JC, Bartels S, Besteiro MAG, Shahollari B, Ulm R, Peck SC. *Arabidopsis* MAP kinase phosphatase 1 (AtMKP1) negatively regulates MPK6-mediated PAMP responses and resistance against bacteria. Plant J. 2011;67:258–68. https://doi.org/10.1111/j.1365-313x.2011.04588.x.

Anderson JC, Wan Y, Kim YM, Pasa-Tolic L, Metz TO, Peck SC. Decreased abundance of type III secretion system-inducing signals in *Arabidopsis* MKP1 enhances resistance against *Pseudomonas syringae*. Proc Nat Acad Sci. 2014;111:6846–51. https://doi.org/10.1073/pnas.1403248111.

Andrade MO, Farah CS, Wang N. The post-transcriptional regulator RsmA/CsrA activates T3SS by stabilizing the 5′ UTR of *hrpG*, the master regulator of *hrp/hrc* genes, in *Xanthomonas*. PLoS Path. 2014;10(2):e1003945. https://doi.org/10.1371/journal.ppat.1003945.

Arnoldo A, Curak J, Kittanakom S, Chevelev I, Lee VT, Sahebol-Amri M, Koscik B, Ljuma L, Roy PJ, Bedalov A, et al. Identification of small molecule inhibitors of *Pseudomonas aeruginosa* exoenzyme S using a yeast phenotypic screen. PLoS Genet. 2008;4:e1000005. https://doi.org/10.1371/journal.pgen.1000005.

Asif M, Xie X, Zhao Z. Virulence regulation in plant-pathogenic bacteria by host-secreted signals. Microbiol Res. 2024a;288:127883. https://doi.org/10.1016/j.micres.2024.127883.

Asif M, Liang S, RenJian H, Xie X, Zhao Z. A stitch in time: sustainable and eco-friendly solutions for kiwifruit bacterial canker. Physiol Mol Plant Pathol. 2024b;136:102506. https://doi.org/10.1016/j.pmpp.2024.102506.

Baltrus DA, McCann HC, Guttman DS. Evolution, genomics and epidemiology of *Pseudomonas syringae*: challenges in bacterial molecular plant pathology. Mol Plant Pathol. 2017;18:152–68. https://doi.org/10.1111/mpp.12506.

Beier D, Gross R. Regulation of bacterial virulence by two-component systems. Curr Opin Microb. 2006;9:143–52. https://doi.org/10.1016/j.mib.2006.01.005.

Bergeron JR. A refined model of the prototypical *Salmonella* SPI-1 T3SS basal body reveals the molecular basis for its assembly. PLoS Path. 2013;9:e1003307. https://doi.org/10.1371/journal.ppat.1003307.

Bergeron JR. The structure of a type 3-secretion system (T3SS) ruler protein suggests a molecular mechanism for needle length sensing. J Biol Chem. 2016;391:1676–91. https://doi.org/10.1074/jbc.M115.684423.

Bergeron JR, Worrall LJ, Sgourakis NG, DiMaio F, Pfuetzner RA, Felise HB, Vuckovic M, Yu AC, Miller SI, Baker D, Strynadka NC. A refined model of the prototypical *Salmonella* SPI-1 T3SS basal body reveals the molecular basis for its assembly. PLoS Path. 2013;9(4):e1003307. https://doi.org/10.1371/journal.ppat.1003307.

Bergeron JRC, Worrall LJ, De S, Sgourakis NG, Cheung AH, Lameignere E, Okon M, Wasney GA, Baker D, McIntosh LP, Strynadka NCJ. The modular structure of the inner-membrane ring component PrgK facilitates assembly of the type III secretion system basal body. Struct. 2015;23(1):161–72. https://doi.org/10.1016/j.str.2014.10.021.

Bernal I, Börnicke J, Heidemann J, Svergun DI, Tuukkanen AT, Uetrecht C, Kolbe M. Molecular organization of soluble type III secretion system sorting platform complexes. J Mol Biol. 2019;431(19):3787–803. https://doi.org/10.1016/j.jmb.2019.07.004.

Brencic A, Winans SC. Detection of and response to signals involved in host-microbe interactions by plant-associated bacteria. Microb Mol Biol Rev. 2005;69:155–94. https://doi.org/10.1128/mmbr.69.1.155-194.2005.

Brencic A, McFarland KA, McManus HR, Castang S, Mogno I, Dove SL, Lory S. The GacS/GacA signal transduction system of *Pseudomonas aeruginosa* acts exclusively through its control over the transcription of the RsmY and RsmZ regulatory small RNAs. Mol Microb. 2009;73:434–45. https://doi.org/10.1111/j.1365-2958.2009.06782.x.

Burkinshaw BJ. Structural analysis of a specialized type III secretion system peptidoglycan-cleaving enzyme. J Biol Chem. 2015;290:10406–17. https://doi.org/10.1074/jbc.M115.639013.

Burkinshaw BJ, Strynadka NCJ. Assembly and structure of the T3SS. Biochim Biophys Acta. 2014;1843(8):1649–63. https://doi.org/10.1016/j.bbamcr.2014.01.035.

Büttner D. Protein export according to schedule: architecture, assembly, and regulation of type III secretion systems from plant- and animal-pathogenic bacteria. Microbiol Mol Biol Rev. 2012;76:262–310. https://doi.org/10.1128/MMBR.05017-11.

Büttner D. Behind the lines—actions of bacterial type III effector proteins in plant cells. FEMS Microbiol Rev. 2016;40:894–937. https://doi.org/10.1093/femsre/fuw026.

Chang JH, Desveaux D, Creason AL. The ABCs and 123s of bacterial secretion systems in plant pathogenesis. Ann Rev Phytopathol. 2014;52:317–45. https://doi.org/10.1146/annurev-phyto-011014-015624.

Charova SN, Gazi AD, Mylonas E, Pozidis C, Sabarit B, Anagnostou D, Psatha K, Aivaliotis M, Beuzon CR, Panopoulos NJ, Kokkinidis M. Migration of type III secretion system transcriptional regulators links gene expression to secretion. Mbio. 2018;9:10–1128. https://doi.org/10.1128/mbio.01096-18.

Chatterjee A, Cui Y, Yang H, Collmer A, Alfano JR, Chatterjee AK. GacA, the response regulator of a two-component system, acts as a master regulator in *Pseudomonas syringae* pv. *tomato* DC3000 by controlling regulatory RNA, transcriptional activators, and alternate sigma factors. Mol Plant Microbe Interact. 2003;16:1106–17. https://doi.org/10.1094/mpmi.2003.16.12.1106.

Chatterjee A, Cui Y, Hasegawa H, Chatterjee AK. PsrA, the *Pseudomonas sigma* regulator, controls regulators of epiphytic fitness, quorum-sensing signals, and plant interactions in *Pseudomonas syringae* pv. *tomato* strain DC3000. Appl Environ Microbiol. 2007;73:3684–94. https://doi.org/10.1128/aem.02445-06.

Chatterjee S, Zhong D, Nordhues BA, Battaile KP, Lovell S, De Guzman RN. The crystal structures of the Salmonella type III secretion system tip protein SipD in complex with deoxycholate and chenodeoxycholate. Protein Sci. 2011;20(1):75–86. https://doi.org/10.1002/pro.537.

Cherradi Y. Interplay between predicted inner-rod and gatekeeper in controlling substrate specificity of the type III secretion system. Mol Microbiol. 2013;87:1183–99. https://doi.org/10.1111/mmi.12158.

Chieda Y, Iiyama K, Yasunaga-Aoki C, Lee JM, Kusakabe T, Shimizu S. Pathogenicity of *gacA* mutant of *Pseudomonas aeruginosa* PA01 in the silkworm. Bombyx Mori FEMS Microbiol Lett. 2005;244:181–6. https://doi.org/10.1016/j.femsle.2005.01.032.

Clarke CR, Cai R, Studholme DJ, Guttman DS, Vinatzer BA. Pseudomonas syringae strains naturally lacking the classical *P. syringae* hrp/hrc locus are common leaf colonizers equipped with an atypical type III secretion system. Mol Plant Microbe Interact. 2010;23:198–210. https://doi.org/10.1094/mpmi-23-2-0198.

Coburn B, Sekirov I, Finlay BB. Type III secretion systems and disease. Clin Microbiol Rev. 2007;20(4):535–49. https://doi.org/10.1128/cmr.00013-07.

Coll NS, Valls M. Current knowledge on the *Ralstonia solanacearum* type III secretion system. Microb Biotechnol. 2013;6:614–20. https://doi.org/10.1111/1751-7915.12056.

Costa TR, Felisberto-Rodrigues C, Meir A, Prevost MS, Redzej A, Trokter M, Waksman G. Secretion systems in Gram-negative bacteria: structural and mechanistic insights. Nat Rev Microbiol. 2015;13:343–59. https://doi.org/10.1038/nrmicro3456.

Crabill E, Karpisek A, Alfano JR. The *Pseudomonas syringae* HrpJ protein controls the secretion of type III translocator proteins and has a virulence role inside plant cells. Mol Microbiol. 2012;85:225–38. https://doi.org/10.1111/j.1365-2958.2012.08097.x.

Davidsson P, Kariola T, Niemi O, Palva E. Pathogenicity of and plant immunity to soft rot pectobacteria. Front Plant Sci. 2013;4:191. https://doi.org/10.3389/fpls.2013.00191.

Davidsson P, Broberg M, Kariola T, Sipari N, Pirhonen M, Palva ET. Short oligogalacturonides induce pathogen resistance-associated gene expression in *Arabidopsis thaliana*. BMC Plant Biol. 2017;17(1):19. https://doi.org/10.1186/s12870-016-0959-183.

Deberdt P, Davezies I, Coranson-Beaudu R, Jestin A. Efficacy of leaf oil from *Pimenta racemosa* var. *racemosa* in controlling bacterial wilt of tomato. Plant Dis. 2018;102:124–31.

Deng X, Lan L, Xiao Y, Kennelly M, Zhou JM, Tang X. *Pseudomonas syringae* two-component response regulator RhpR regulates promoters carrying an inverted repeat element. Mol Plant Microbe Interact. 2010;23:927–39. https://doi.org/10.1094/mpmi-23-7-0927.

Deng X, Liang H, Chen K, He C, Lan L, Tang X. Molecular mechanisms of two-component system RhpRS regulating type III secretion system in *Pseudomonas syringae*. Nucl Acids Res. 2014;42:11472–86. https://doi.org/10.1093/nar/gku865.

Deng W, Marshall NC, Rowland JL, McCoy JM, Worrall LJ, Santos AS, Strynadka NCJ, Finlay BB. Assembly, structure, function and regulation of type III secretion systems. Nat Rev Microbiol. 2017;15:323–37. https://doi.org/10.1038/nrmicro.2017.20.

Diepold A, Wagner S. Assembly of the bacterial type III secretion machinery. FEMS Microbiol Rev. 2014;38:802–22. https://doi.org/10.1111/1574-6976.12061.

Diepold A, Kudryashev M, Delalez NJ, Berry RM, Armitage P. Composition, formation, and regulation of the cytosolic C-ring, a dynamic component of the type III secretion injectisome. PLoS Biol. 2015;13:e1002039. https://doi.org/10.1371/journal.pbio.1002039.

Djami-Tchatchou AT, Harrison GA, Harper CP, Wang R, Prigge MJ, Estelle M, Kunkel BN. (2019) Dual role of auxin in regulating plant defense and bacterial virulence gene expression during *Pseudomonas syringae* Pto DC3000 pathogenesis. Mol Plant Microbe Interact. 2020;33(8):1059–71. https://doi.org/10.1094/MPMI-02-20-0047-R.

Du J. The type III secretion system apparatus determines the intracellular niche of bacterial pathogens. Proc Natl Acad Sci. 2016;113:4794–9. https://doi.org/10.1073/pnas.1520699113.

Duncan MC, Wong WR, Dupzyk AJ, Bray WM, Linington RG, Auerbuch V. An NF-κB-based high-throughput screen identifies piericidins as inhibitors of the *Yersinia pseudotuberculosis* type III secretion system. Antimicrob Agents Chemother. 2014;58:1118–26. https://doi.org/10.1128/aac.02025-13.

Fan S, Tian F, Li J, Hutchins W, Chen H, Yang F, Yuan X, Cui Z, Yang CH, He C. Identification of phenolic compounds that suppress the virulence of *Xanthomonas oryzae* on rice via the type III secretion system. Mol Plant Pathol. 2017;18:555–68. https://doi.org/10.1111/mpp.12415.

Fan S, Tian F, Fang L, Yang CH, He C. Transcriptional responses of *Xanthomonas oryzae* pv. *oryzae* to type III secretion system inhibitor ortho-coumaric acid. BMC Microbiol. 2019;19:163. https://doi.org/10.1186/s12866-019-1532-5.

Felise HB, Nguyen HV, Pfuetzner RA, Barry KC, Jackson SR, Blanc MP, Bronstein PA, Kline T, Miller SI. An inhibitor of gram-negative bacterial virulence protein secretion. Cell Host Microbe. 2008;4(4):325–336

Ferreira AO, Myers CR, Gordon JS, Martin GB, Vencato M, Collmer A, Wehling MD, Alfano JR, Moreno-Hagelsieb G, Lamboy WF, et al. Whole-genome expression profiling defines the hrpL regulon of *Pseudomonas syringae* pv. *tomato* DC3000, allows de novo reconstruction of the hrp cis element, and identifies novel coregulated genes. Mol Plant Microbe Interact. 2006;19:1167–79. https://doi.org/10.1094/mpmi-19-1167.

Ferreiro MD, Nogales J, Farias GA, Olmedilla A, Sanjuán J, Gallegos MT. Multiple CsrA proteins control key virulence traits in *Pseudomonas syringae* pv. *tomato* DC3000. Mol Plant Microbe Interact. 2018;31:525–36. https://doi.org/10.1094/mpmi-09-17-0232-r.

Fishman MR, Filiatrault MJ. Prevention of surface-associated calcium phosphate by the *Pseudomonas syringae* two-component system CvsSR. J Bacteriol. 2019;201:10–1128. https://doi.org/10.1128/jb.00584-18.

Fishman MR, Zhang J, Bronstein PA, Stodghill P, Filiatrault MJ. Ca2+ -induced two-component system CvsSR regulates the type III secretion system and the extracytoplasmic function sigma factor AlgU in *Pseudomonas syringae* pv. *tomato* DC3000. J Bacteriol. 2018;2018:200. https://doi.org/10.1128/jb.00538-17.

Fouts DE, Abramovitch RB, Alfano JR, Baldo AM, Buell CR, Cartinhour S, Chatterjee AK, D'Ascenzo M, Gwinn ML, Lazarowitz SG, et al. Genomewide identification of *Pseudomonas syringae* pv. *tomato* DC3000 promoters controlled by the HrpL alternative sigma factor. Proc Natl Acad Sci. 2002;99:2275–80. https://doi.org/10.1073/pnas.032514099.

Fu ZQ, Guo M, Alfano JR. *Pseudomonas syringae* HrpJ is a type III secreted protein that is required for plant pathogenesis, injection of effectors, and secretion of the HrpZ1 harpin. J Bacteriol. 2006;188:6060–9. https://doi.org/10.1128/jb.00718-06.

Fujii T, Cheung M, Blanco A, Kato T, Blocker AJ, Namba K. Structure of a type III secretion needle at 7-Å resolution provides insights into its assembly and signaling mechanisms. Proc Nat Acad Sci. 2012;109(12):4461–6. https://doi.org/10.1073/pnas.1116126109.

Gaytán MO, Martínez-Santos VI, Soto E, González-Pedrajo B. Type three secretion system in attaching and effacing pathogens. Front Cell Infect Microbiol. 2016;6:129. https://doi.org/10.3389/fcimb.2016.00129.

Gazi AD, Sarris PF, Fadouloglou VE, Charova SN, Mathioudakis N, Panopoulos NJ, Kokkinidis M. Phylogenetic analysis of a gene cluster encoding an additional, rhizobial-like type III secretion system that is narrowly distributed among *Pseudomonas syringae* strains. BMC Microbiol. 2012;12:188. https://doi.org/10.1186/1471-2180-12-188.

Gill EE, Franco OL, Hancock REW. Antibiotic adjuvants: diverse strategies for controlling drug-resistant pathogens. Chem Biol Drug Design. 2015;85:56–78. https://doi.org/10.1111/cbdd.12478.

González-Fuente M, Carrère S, Monachello D, Marsella BG, Cazalé AC, Zischek C, Mitra RM, Rezé N, Cottret L, Mukhtar MS, Lurin C, Noël LD, Peeters N. EffectorK, a comprehensive resource to mine for *Ralstonia*, *Xanthomonas*, and other published effector interactors in the *Arabidopsis* proteome. Mol Plant Pathol. 2020;21(10):1257–70. https://doi.org/10.1111/mpp.12965.

Grier MC, Garrity-Ryan LK, Bartlett VJ, Klausner KA, Donovan PJ, Dudley C, Alekshun MN, Tanaka SK, Draper MP, Levy SB, Kim OK. N-Hydroxybenzimidazole inhibitors of ExsA MAR transcription factor in *Pseudomonas aeruginosa*: in vitro anti-virulence activity and metabolic stability. Bioorg

Med Chem Lett. 2010;20:3380–3. https://doi.org/10.1016/j.bmcl.2010.04.014.
Gupta G, Chauhan PS, Jha PN, et al. Secretory molecules from secretion systems fine-tune the host-beneficial bacteria (PGPRs) interaction. Front Microbiol. 2024;15:1355750.
Haapalainen M, van Gestel K, Pirhonen M, Taira S. Soluble plant cell signals induce the expression of the type III secretion system of *Pseudomonas syringae* and upregulate the production of pilus protein HrpA. Mol Plant Microbe Interact. 2009;22:282–90. https://doi.org/10.1094/mpmi-22-3-0282.
Han S, Yang L, Wang Y, Ran Y, Li S, Ding W. Preliminary studies on the antibacterial mechanism of a new plant-derived compound, 7-methoxycoumarin, against *Ralstonia solanacearum*. Front Microbiol. 2021;12:697911. https://doi.org/10.3389/fmicb.2021.697911.
He LL, Wang X, Rothenberg DON, Xu X, Wang HH, Deng X, Cui ZN. A novel strategy to control *Pseudomonas syringae* through inhibition of type III secretion system. Pest Biochem Physiol. 2023;194:105471. https://doi.org/10.1016/j.pestbp.2023.105471.
Heeb S, Haas D. Regulatory roles of the GacS/GacA two-component system in plant-associated and other gram-negative bacteria. Mol Plant Microbe Interact. 2001;14:1351–63. https://doi.org/10.1094/mpmi.2001.14.12.1351.
Heeb S, Blumer C, Haas D. Regulatory RNA as mediator in GacA/RsmA-dependent global control of exoproduct formation in *Pseudomonas fluorescens* CHA0. J Bacteriol. 2002;184:1046–56. https://doi.org/10.1128/jb.184.4.1046-1056.2002.
Helmann TC, Deutschbauer AM, Lindow SE. Genome-wide identification of *Pseudomonas syringae* genes required for fitness during colonization of the leaf surface and apoplast. Proc Natl Acad Sci. 2019;116:18900–10. https://doi.org/10.1073/pnas.1908858116.
Hendrickson EL, Guevera P, Ausubel FM. The alternative sigma factor RpoN is required for hrp activity in *Pseudomonas syringae* pv. *mculicola* and acts at the level of HrpL transcription. J Bacteriol. 2000;182:3508–16. https://doi.org/10.1128/jb.182.12.3508-3516.2000.
Hu B, Morado DR, Margolin W, et al. Visualization of the type III secretion sorting platform of *Shigella flexneri*. Proc Natl Acad Sci. 2015;112(4):1047–52. https://doi.org/10.1073/pnas.1411610112.
Huang YC, Lin YC, Wei CF, Deng WL, Huang HC. The pathogenicity factor HrpF interacts with HrpA and HrpG to modulate type III secretion system (T3SS) function and T3SS expression in *Pseudomonas syringae* pv. *averrhoi*. Mol Plant Pathol. 2016;17:1080–94. https://doi.org/10.1111/mpp.12349.
Huang J, Yao C, Sun Y, Ji Q, Deng X. Virulence-related regulatory network of *Pseudomonas syringae*. Struct Biotechnol J. 2022;20:6259–70. https://doi.org/10.1016/j.csbj.2022.11.011.
Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev. 1998;62(2):379–433. https://doi.org/10.1128/MMBR.62.2.379-433.1998.
Hutcheson SW, Bretz J, Sussan T, Jin S, Pak K. Enhancer-binding proteins HrpR and HrpS interact to regulate *hrp*-encoded type III protein secretion in *Pseudomonas syringae* strains. J Bacteriol. 2001;183:5589–98. https://doi.org/10.1128/jb.183.19.5589-5598.2001.
Jacobs JM, Pesce C, Lefeuvre P, Koebnik R. Comparative genomics of a cannabis pathogen reveals insight into the evolution of pathogenicity in *Xanthomonas*. Front Plant Sci. 2015;6:431.
Jessen DL, Bradley DS, Nilles ML. A type III secretion system inhibitor targets YopD while revealing differential regulation of secretion in calcium-blind mutants of *Yersinia pestis*. Antimicrob Agents Chemother. 2014;58:839–50. https://doi.org/10.1128/aac.01170-13.
Jiang S, Li H, Ahmed W, Xiang X, Song G, Cui ZN. Discovery of ethyl 2-nitro-3-arylacrylates molecules as T3SS inhibitor reducing the virulence of plant pathogenic bacteria *Xanthomonas*. Front Microbiol. 2019;10:1874. https://doi.org/10.3389/fmicb.2019.01874.
Journet L, Agrain C, Broz P, Cornelis GR. The needle length of bacterial injectisomes is determined by a molecular ruler. Science. 2003;302(5651):1757–60. https://doi.org/10.1126/science.1091422.
Jovanovic M, James EH, Burrows PC, Rego FGM, Buck M, Schumacher J. Regulation of the co-evolved HrpR and HrpS AAA+ proteins required for *Pseudomonas syringae* pathogenicity. Nat Commun. 2011;2:177. https://doi.org/10.1038/ncomms1177.
Jovanovic M, Lawton E, Schumacher J, Buck M. Interplay among *Pseudomonas syringae* HrpR, HrpS and HrpV proteins for regulation of the type III secretion system. FEMS Microbiol Lett. 2014;356:201–11. https://doi.org/10.1111/1574-6968.12476.
Jung K, Fried L, Behr S, Heermann R. Histidine kinases and response regulators in networks. Curr Opin Microbiol. 2012;15(2):118–24. https://doi.org/10.1016/j.mib.2011.11.009.
Kang JE, Jeon BJ, Park MY, Yang HJ, Kwon J, Lee DH, Kim BS. Inhibition of the type III secretion system of *Pseudomonas syringae* pv. *tomato* DC3000 by resveratrol oligomers identified in *Vitis vinifera* L. Pest Manag Sci. 2020;76:2294–303. https://doi.org/10.1002/ps.5764.
Khokhani D, Zhang C, Li Y, Wang Q, Zeng Q, Yamazaki A, Hutchins W, Zhou SS, Chen X, Yang CH. Discovery of plant phenolic compounds that act as type III secretion system inhibitors or inducers of the fire blight pathogen *Erwinia amylovora*. Appl Environ Microbiol. 2023;79:5424–36. https://doi.org/10.1128/aem.00845-13.
Kline T, Barry KC, Jackson SR, Felise HB, Nguyen HV, Miller SI. Tethered thiazolidinone dimers as inhibitors of the bacterial type III secretion system. Bioorg Med Chem Lett. 2009;19:1340–3. https://doi.org/10.1016/j.bmcl.2009.01.047.
Koretke KK, Lupas AN, Warren PV, Rosenberg M, Brown JR. Evolution of two-component signal transduction. Mol Biol Evol. 2000;17:1956–70. https://doi.org/10.1093/oxfordjournals.molbev.a026297.
Kowal J. Structure of the dodecameric *Yersinia enterocolitica* secretin YscC and its trypsin-resistant core. Structure. 2013;21:2152–61. https://doi.org/10.1016/j.str.2013.09.012.
Kumar V, Sharma A, Kaur R, Thukral AK, Bhardwaj R, Ahmad P. Differential distribution of amino acids in plants. Amino Acids. 2017;49:821–69. https://doi.org/10.1007/s00726-017-2401-x.
Lan L, Deng X, Zhou J, Tang X. Genome-wide gene expression analysis of *Pseudomonas syringae* pv. *tomato* DC3000 reveals overlapping and distinct pathways regulated by hrpL and hrpRS. Mol Plant Microbe Interact. 2006;19:976–87. https://doi.org/10.1094/mpmi-19-0976.
Lan L, Deng X, Xiao Y, Zhou J-M, Tang X. Mutation of Lon protease differentially affects the expression of *Pseudomonas syringae* type III secretion system genes in rich and minimal media and reduces pathogenicity. Mol Plant Microbe Interact. 2007;20:682–96. https://doi.org/10.1094/mpmi-20-6-0682.
Landry D, González-Fuente M, Deslandes L, Peeters N. The large, diverse, and robust arsenal of *Ralstonia solanacearum* type III effectors and their in planta functions. Mol Plant Pathol. 2020;21(10):1377–88. https://doi.org/10.1111/mpp.12977.
Latour X. The evanescent GacS signal. Microorganisms. 2020;8:1746. https://doi.org/10.3390/microorganisms8111746.
Lee VT, Pukatzki S, Sato H, Kikawada E, Kazimirova AA, Huang J, Li X, Arm JP, Frank DW, Lory S. Pseudolipasin A is a specific inhibitor for phospholipase A2 activity of *Pseudomonas aeruginosa* cytotoxin ExoU. Infect Immun. 2007;75:1089–98. https://doi.org/10.1128/iai.01184-06.
Lee D, Bourdais G, Yu G, Robatzek S, Coaker G. Phosphorylation of the plant immune regulator RPM1-interacting protein4 enhances plant plasma membrane $H^+$-ATPase activity and inhibits flagellin-triggered immune responses in *Arabidopsis*. Plant Cell. 2015;27:2042–56. https://doi.org/10.1105/tpc.114.132308.
Lefebre MD, Galán JE. The inner rod protein controls substrate switching and needle length in a *Salmonella* type III secretion system. Proc Natl Acad Sci. 2014;111:817–22. https://doi.org/10.1073/pnas.1319698111.
Li YR, Zou HS, Che YZ, Cui YP, Guo W, Zou LF, Chatterjee S, Biddle EM, Yang CH, Chen GY. A novel regulatory role of HrpD6 in regulating hrp-hrc-hpa genes in *Xanthomonas oryzae* pv. *oryzicola*. Mol Plant Microbe Interact. 2011;24:1086–101. https://doi.org/10.1094/mpmi-09-10-0205.
Li Y, Hutchins W, Wu X, Liang C, Zhang C, Yuan X, Khokhani D, Chen X, Che Y, Wang Q, Yang CH. Derivative of plant phenolic compound inhibits the type III secretion system of *Dickeya dadantii* via HrpX/HrpY two-component signal transduction and Rsm systems. Mol Plant Pathol. 2015;16:150–63. https://doi.org/10.1111/mpp.12168.
Li S, Pi J, Zhu H, Yang L, Zhang X, Ding W. Caffeic acid in tobacco root exudate defends tobacco plants from infection by *Ralstonia solanacearum*. Front Plant Sci. 2021;12:690586.
Lindeberg M, Cartinhour S, Myers CR, Schechter LM, Schneider DJ, Collmer A. Closing the circle on the discovery of genes encoding *hrp* regulon members and type III secretion system effectors in the genomes of

three model *Pseudomonas syringae* strains. Mol Plant Microbe Interact. 2006;19:1151–8. https://doi.org/10.1094/mpmi-19-1151.
Lipscomb L, Schell MA. Elucidation of the regulon and cis-acting regulatory element of HrpB, the AraC-type regulator of a plant pathogen-like type III secretion system in *Burkholderia pseudomallei*. J Bacteriol. 2011;193:1991–2001. https://doi.org/10.1128/jb.01379-10.
Lorenzini E, Singer A, Singh B, Lam R, Skarina T, Chirgadze NY, Savchenko A, Gupta RS. Structure and protein-protein interaction studies on *Chlamydia trachomatis* protein CT670 (YscO Homolog). J Bacteriol. 2010;192(11):2746–56. https://doi.org/10.1128/JB.01479-09.
Lowe-Power TM, Jacobs JM, Ailloud F, Fochs B, Prior P, Allen C. Degradation of the plant defense signal salicylic acid protects *Ralstonia solanacearum* from toxicity and enhances virulence on tobacco. Mbio. 2016;7(3):e00656-16. https://doi.org/10.1128/mBio.00656-16.
Lowe-Power TM, Khokhani D, Allen C. How *Ralstonia solanacearum* exploits and thrives in the flowing plant xylem environment. Trends Microbiol. 2018;26(11):929–42. https://doi.org/10.1016/j.tim.2018.06.002.
Lv H, Zhang Y, Shao J, Liu H, Wang Y. Ferulic acid production by metabolically engineered *Escherichia coli*. Biores Bioproc. 2021;8(1):70.
Ma YN, Chen L, Si NG, Jiang WJ, Zhou ZG, Liu JL, Zhang LQ. Identification of benzyloxy carbonimidoyl dicyanide derivatives as novel type III secretion system inhibitors via high-throughput screening. Front Plant Sci. 2019;10:1059. https://doi.org/10.3389/fpls.2019.01059.
MacLean D, Studholme DJ. A boolean model of the *Pseudomonas syringae hrp* regulon predicts a tightly regulated system. PLoS ONE. 2010;5:e9101. https://doi.org/10.1371/journal.pone.0009101.
Macnab RM. How bacteria assemble flagella. Annu Rev Microbiol. 2003;57:77–100. https://doi.org/10.1146/annurev.micro.57.030502.090832.
Makino F, Shen D, Kajimura N, Kawamoto A, Pissaridou P, Oswin H, Pain M, Murillo I, Namba K, Blocker AJ. The architecture of the cytoplasmic region of type III secretion systems. Sci Rep. 2016;6:33341. https://doi.org/10.1038/srep33341.
Marsden AE, King JM, Spies MA, Kim OK, Yahr TL. Inhibition of *Pseudomonas aeruginosa* ExsA DNA-binding activity by N-Hydroxybenzimidazoles. Antimicrob Agents Chemother. 2016;60:766–76. https://doi.org/10.1128/aac.02242-15.
Marutani M, Taguchi F, Ogawa Y, Hossain MM, Inagaki Y, Toyoda K, Shiraishi T, Ichinose Y. Gac two-component system in *Pseudomonas syringae* pv. *tabaci* is required for virulence but not for hypersensitive reaction. Mol Genet Gen. 2007;279:313–22. https://doi.org/10.1007/s00438-007-0309-y.
McAtee PA, Brian L, Curran B, van der Linden O, Nieuwenhuizen NJ, Chen X, Henry-Kirk RA, Stroud EA, Nardozza S, Jayaraman J, Rikkerink EHA, Print CG, Allan AC, Templeton MD. Re-programming of *Pseudomonas syringae* pv. *actinidiae* gene expression during early stages of infection of kiwifruit. BMC Gen. 2018;19(1):822. https://doi.org/10.1186/s12864-018-5197-5.
McClerklin SA, Lee SG, Harper CP, Nwumeh R, Jez JM, Kunkel BN. Indole-3-acetaldehyde dehydrogenase-dependent auxin synthesis contributes to virulence of *Pseudomonas syringae* strain DC3000. PLoS Path. 2018;14(1):e1006811. https://doi.org/10.1371/journal.ppat.1006811.
McDermott JE. Computational prediction of type III and IV secreted effectors in Gram-negative bacteria. Infect Immun. 2011;79:23–32. https://doi.org/10.1128/IAI.00537-10.
Merda D, Briand M, Bosis E, Rousseau C, Portier P, Barret M, Jacques MA, Fischer-Le SM. Ancestral acquisitions, gene flow and multiple evolutionary trajectories of the type three secretion system and effectors in *Xanthomonas* plant pathogens. Mol Ecol. 2017;26(21):5939–52. https://doi.org/10.1111/mec.14343.
Mo YY, Gross DC. Plant signal molecules activate the syrB gene, which is required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. J Bacteriol. 1991;173:5784–92. https://doi.org/10.1128/jb.173.18.5784-5792.1991.
Moll S, Schneider DJ, Stodghill P, Myers CR, Cartinhour SW, Filiatrault MJ. Construction of an RsmX co-variance model and identification of five rsmX non-coding RNAs in *Pseudomonas syringae* pv. *tomato* DC3000. RNA Biol. 2010;7:508–16. https://doi.org/10.4161/rna.7.5.12687.
Murillo I, Martinez-Argudo I, Blocker AJ. Genetic dissection of the signaling cascade that controls activation of the *Shigella* type III secretion system from the needle tip. Sci Rep. 2016;6:27649. https://doi.org/10.1038/srep27649.
Negrea A, Bjur E, Ygberg SE, Elofsson M, Wolf-Watz H, Rhen M. Salicylidene acylhydrazides that affect type III protein secretion in *Salmonella enterica* serovar *Typhimurium*. Antimicrob Agents Chem. 2007;51:2867–76. https://doi.org/10.1128/aac.00223-07.
Ngo TD, Plé S, Thomas A, Barette C, Fortuné A, Bouzidi Y, Fauvarque MO, Pereira de Freitas R, Francisco Hilário F, Attree I, Wong YS. Chimeric protein–protein interface inhibitors allow efficient inhibition of type III secretion machinery and *Pseudomonas aeruginosa* virulence. ACS Infect Dis. 2019;5(11):1843–54.
Notti RQ, Stebbins CE. The structure and function of type III secretion systems. Microbiol Spec. 2016;4:241–64. https://doi.org/10.1128/microbiolspec.vmbf-0004-2015.
Notti RQ, Bhattacharya S, Lilic M, Stebbins CE. A common assembly module in injectisome and flagellar type III secretion sorting platforms. Nat Commun. 2015;7:7125. https://doi.org/10.1038/ncomms8125.
O'Malley MR, Anderson JC. Regulation of the *Pseudomonas syringae* type III secretion system by host environment signals. Microorganisms. 2021;9(6):1227. https://doi.org/10.3390/microorganisms9061227.
O'Malley MR, Chien CF, Peck SC, Lin NC, Anderson JC. A revised model for the role of GacS/GacA in regulating type III secretion by *Pseudomonas syringae* pv. *tomato* DC3000. Mol Plant Pathol. 2019;21:139–44. https://doi.org/10.1111/mpp.12876.
Occhialini A, Cunnac S, Reymond N, Genin S, Boucher C. Genome-wide analysis of gene expression in *Ralstonia solanacearum* reveals that the *hrpB* gene acts as a regulatory switch controlling multiple virulence pathways. Mol Plant Microbe Interact. 2005;18(9):938–49.
Ortiz-Martín I, Thwaites R, Macho AP, Mansfield JW, Beuzón CR. Positive regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*. Mol Plant Microbe Interact. 2010a;23:665–81. https://doi.org/10.1094/mpmi-23-5-0665.
Ortiz-Martín I, Thwaites R, Mansfield JW, Beuzón CR. Negative regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*. Mol Plant Microbe Interact. 2010b;23:682–701. https://doi.org/10.1094/mpmi-23-5-0682.
Peeters N, Carrère S, Anisimova M, Plener L, Cazalé AC, Genin S. Repertoire, unified nomenclature and evolution of the type III effector gene set in the *Ralstonia solanacearum* species complex. BMC Genom. 2013;14:859. https://doi.org/10.1186/1471-2164-14-859.
Pernestig AK, Melefors Ö, Georgellis D. Identification of UvrY as the cognate response regulator for the BarA sensor kinase in *Escherichia coli*. J Biol Chem. 2001;276:225–31. https://doi.org/10.1074/jbc.m001550200.
Portaliou AG, Tsolis KC, Loos MS, Zorzini V, Economou A. Type III secretion: building and operating a remarkable nanomachine. Trends Biochem Sci. 2016;41:175–89. https://doi.org/10.1016/j.tibs.2015.09.005.
Pradhanang PM, Momol MT, Olson SM, Jones JB. Effects of plant essential oils on *Ralstonia solanacearum* population density and bacterial wilt incidence in tomato. Plant Dis. 2003;87(4):423–7. https://doi.org/10.1094/PDIS.2003.87.4.423.
Puigvert M, Solé M, López-Garcia B, Coll NS, Beattie KD, Davis RA, Elofsson M, Valls M. Type III secretion inhibitors for the management of bacterial plant diseases. Mol Plant Pathol. 2021;20:20–32. https://doi.org/10.1111/mpp.12736.
Qi P, Huang M, Hu X, Zhang Y, Wang Y, Li P, Chen S, Zhang D, Cao S, Zhu W, Xie J, Cheng J, Fu Y, Jiang D, Yu X, Li B. A *Ralstonia solanacearum* effector targets TGA transcription factors to subvert salicylic acid signaling. Plant Cell. 2022;34(5):1666–83. https://doi.org/10.1093/plcell/koac015.
Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, Liang H, Song X, Wu M. *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduct Target Ther. 2022;7:199. https://doi.org/10.1038/s41392-022-01056-1.
Ramos AR, Morello JE, Ravindran S, Deng WL, Huang HC, Collmer A. Identification of *Pseudomonas syringae* pv. *syringae* 61 type III secretion system Hrp proteins that can travel the type III pathway and contribute to the translocation of effector proteins into plant cells. J Bacteriol. 2007;189:5773–8. https://doi.org/10.1128/jb.00435-07.
Raveau R, Fontaine J, Lounès-Hadj SA. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: a review. Foods. 2020;9(3):365. https://doi.org/10.3390/foods9030365.
Rico A, Preston GM. *Pseudomonas syringae* pv. *tomato* DC3000 uses constitutive and apoplast-induced nutrient assimilation pathways to catabolize

nutrients that are abundant in the tomato apoplast. Mol Plant Microbe Interact. 2008;21:269–82. https://doi.org/10.1094/mpmi-21-2-0269.
Rosqvist R, Magnusson KE, Wolf-Watz H. Target cell contact triggers expression and polarized transfer of *Yersinia* YopE cytotoxin into mammalian cells. EMBO J. 1994;13(4):964–72. https://doi.org/10.1002/j.1460-2075.1994.tb06341.x.
Sankhe GD, Raja R, Singh DP, Bheemireddy S, Rana S, Athira PJ, Dixit NM, Saini DK. Sequestration of histidine kinases by non-cognate response regulators establishes a threshold level of stimulation for bacterial two-component signalling. Nat Commun. 2023;14(1):4483. https://doi.org/10.1038/s41467-023-40095-2.
Schechter LM, Vencato M, Jordan KL, Schneider SE, Schneider DJ, Collmer A. Multiple approaches to a complete inventory of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion system effector proteins. Mol Plant Microbe Interact. 2006;19:1180–92. https://doi.org/10.1094/mpmi-19-1180.
Schraidt O, Marlovits TC. Three-dimensional model of Salmonella's needle complex at subnanometer resolution. Science. 2011;331(6021):1192–5. https://doi.org/10.1126/science.1199358.
Schraidt O, Lefebre MD, Brunner MJ, et al. Topology and organization of the *Salmonella typhimurium* type III secretion needle complex components. PLoS Pathog. 2010;6(4):e1000824. https://doi.org/10.1371/journal.ppat.1000824.
Shi Y, Xiong LT, Li H, Li WL, O'Neill Rothenberg D, Liao LS, Deng X, Song GP, Cui ZN. Derivative of cinnamic acid inhibits T3SS of *Xanthomonas oryzae* pv. *oryzae* through the HrpG-HrpX regulatory cascade. Bioorg Chem. 2023;141:106871. https://doi.org/10.1016/j.bioorg.2023.106871.
Singh B, Röhm KH. Characterization of a *Pseudomonas putida* ABC transporter (AatJMQP) required for acidic amino acid uptake: biochemical properties and regulation by the Aau two-component system. Microbiol. 2008;154:797–809. https://doi.org/10.1099/mic.0.2007/013185-0.
Slepenkin A, Chu H, Elofsson M, Keyser P, Peterson EM. Protection of mice from a *Chlamydia trachomatis* vaginal infection using a salicylidene acylhydrazide, a potential microbicide. J Infect Dis. 2011;204:1313–20. https://doi.org/10.1093/infdis/jir552.
Sonawane AM, Singh B, Röhm KH. The AauR-AauS two-component system regulates uptake and metabolism of acidic amino acids in *Pseudomonas putida*. Appl Environ Microbiol. 2006;72:6569–77. https://doi.org/10.1128/aem.00830-06.
Stael S, Wurzinger B, Mair A, Mehlmer N, Vothknecht UC, Teige M. Plant organellar calcium signalling: an emerging field. J Exp Bot. 2011;63:1525–42. https://doi.org/10.1093/jxb/err394.
Sturm A, Heinemann M, Arnoldini M, Benecke A, Ackermann M, Benz M, Dormann J, Hardt WD. The cost of virulence: retarded growth of *Salmonella typhimurium* cells expressing type III secretion system 1. PLoS Path. 2011;7:e1002143. https://doi.org/10.1371/journal.ppat.1002143.
Sun YH, Rolán HG, Tsolis RM. Injection of flagellin into the host cell cytosol by *Salmonella enterica* serotype *typhimurium*. J Biol Chem. 2007;282:33897–901. https://doi.org/10.1074/jbc.C700181200.
Tang X, Xiao Y, Zhou JM. Regulation of the type III secretion system in phytopathogenic bacteria. Mol Plant Microbe Interact. 2006;19:1159–66. https://doi.org/10.1094/mpmi-19-1159.
Tang Y, Romano FB, Breña M, Heuck AP. The *Pseudomonas aeruginosa* type III secretion translocator PopB assists the insertion of the PopD translocator into host cell membranes. Biol Chem. 2018;293(23):8982–93. https://doi.org/10.1074/jbc.RA118.002766.
Tao H, Tian H, Jiang S, Xiang X, Ahmed W, Tang R, Cui ZN. Synthesis and bioactivity of 1,3-thiazolidine-2-thione derivatives against type III secretion system of *Xanthomonas oryzae*. Bioorg Med Chem. 2019;27:3364–71. https://doi.org/10.1016/j.bmc.2019.06.019.
Teulet A, Camuel A, Perret X, Giraud E. The versatile roles of type III secretion systems in rhizobium-legume symbioses. Ann Rev Microbiol. 2022;76:45–65. https://doi.org/10.1146/annurev-micro-041020-032624.
Thomas NA, Ma I, Prasad ME, Rafuse C. Expanded roles for multicargo and class 1B effector chaperones in type III secretion. J Bacteriol. 2012;194(15):3767–73. https://doi.org/10.1128/JB.00406-12.
Thwaites R, Spanu PD, Panopoulos NJ, Stevens C, Mansfield JW. Transcriptional regulation of components of the type III secretion system and effectors in *Pseudomonas syringae* pv. *phaseolicola*. Mol Plant Microbe Interact. 2004;17:1250–8. https://doi.org/10.1094/mpmi.2004.17.11.1250.
Tree JJ, Wang D, McInally C, Mahajan A, Layton A, Houghton I, Elofsson M, Stevens MP, Gally DL, Roe AJ. Characterization of the effects of salicylidene acylhydrazide compounds on type III secretion in *Escherichia coli* O157:H7. Infect Immun. 2009;77:4209–20. https://doi.org/10.1128/iai.00562-09.
Turner SE, Pang YY, O'Malley MR, Weisberg AJ, Fraser VN, Yan Q, Chang JH, Anderson JC. A DeoR-type transcription regulator is required for sugar-induced expression of type III secretion-encoding genes in *Pseudomonas syringae* pv. *tomato* DC3000. Mol Plant Microbe Interact. 2020;33:509–18. https://doi.org/10.1094/mpmi-10-19-0290-r.
Vailleau F, Genin S. *Ralstonia solanacearum*: an arsenal of virulence strategies and prospects for resistance. Annu Rev Phytopathol. 2023;61:25–47. https://doi.org/10.1146/annurev-phyto-021622-104551.
Valentini M, Gonzalez D, Mavridou DAI, Filloux A. Lifestyle transitions and adaptive pathogenesis of *Pseudomonas aeruginosa*. Curr Opin Microbiol. 2018;41:15–20. https://doi.org/10.1016/j.mib.2017.11.006.
Vargas P, Farias GA, Nogales J, Prada H, Carvajal V, Barón M, Rivilla R, Martín M, Olmedilla A, Gallegos MT. Plant flavonoids target *Pseudomonas syringae* pv. *tomato* DC3000 flagella and type III secretion system. Environ Microbiol Rep. 2013;5:841–50. https://doi.org/10.1111/1758-2229.12086.
Vinatzer BA, Teitzel GM, Lee MW, Jelenska J, Hotton S, Fairfax K, Jenrette J, Greenberg JT. The type III effector repertoire of *Pseudomonas syringae* pv. *syringae* B728a and its role in survival and disease on host and non-host plants. Mol Microbiol. 2006;62:26–44. https://doi.org/10.1111/j.1365-2958.2006.05350.x.
Waite C, Schumacher J, Jovanovic M, Bennett M, Buck M. Negative autogenous control of the master type III secretion system regulator HrpL in *Pseudomonas syringae*. Mbio. 2017;8:10–1128. https://doi.org/10.1128/mbio.02273-16.
Wang J, Shao X, Zhang Y, Zhu Y, Yang P, Yuan J, Wang T, Yin C, Wang W, Chen S, et al. HrpS is a global regulator on type III secretion system (T3SS) and non-T3SS genes in *Pseudomonas savastanoi* pv. *phaseolicola*. Mol Plant Microbe Interact. 2018;31:1232–43. https://doi.org/10.1094/mpmi-02-18-0035-r.
Wang W, Yang J, Zhang J, Liu YX, Tian C, Qu B, Gao C, Xin P, Cheng S, Zhang W, et al. An *Arabidopsis* secondary metabolite directly targets expression of the bacterial type III secretion system to inhibit bacterial virulence. Cell Host Microbe. 2020;27:601-613.e607. https://doi.org/10.1016/j.chom.2020.03.004.
Wee DH, Hughes KT. Molecular ruler determines needle length for the *Salmonella* Spi-1 injectisome. Proc Natl Acad Sci. 2015;112(13):4098–103. https://doi.org/10.1073/pnas.1423492112.
Wei W, Plovanich-Jones A, Deng WL, Jin QL, Collmer A, Huang HC, He SY. The gene coding for the Hrp pilus structural protein is required for type III secretion of Hrp and Avr proteins in *Pseudomonas syringae* pv. *tomato*. Proc Nat Acad Sci. 2000;97:2247–52. https://doi.org/10.1073/pnas.040570097.
Wei CF, Deng WL, Huang HC. A chaperone-like HrpG protein acts as a suppressor of HrpV in regulation of the *Pseudomonas syringae* pv. *syringae* type III secretion system. Mol Microbiol. 2005;57:520–36. https://doi.org/10.1111/j.1365-2958.2005.04704.x.
West AH, Stock AM. Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem Sci. 2001;26:369–76. https://doi.org/10.1016/s0968-0004(01)01852-7.
White FF, Potnis N, Jones JB, Koebnik R. The type III effectors of *Xanthomonas*. Mol Plant Pathol. 2009;10(6):749–66. https://doi.org/10.1111/j.1364-3703.2009.00590.x.
Worrall ZR, Deng W, Vuckovic M, Felise HB, Nguyen HV, Miller SI, Finlay BB, Strynadka NC. Structural analysis of the essential self-cleaving type III secretion proteins EscU and SpaS. Nature. 2008;453(7191):124–7. https://doi.org/10.1038/nature06832.
Worrall LJ, Hong C, Vuckovic M, Deng W, Bergeron JRC, Majewski DD, Huang RK, Spreter T, Finlay BB, Yu Z, Strynadka NCJ. Near-atomic-resolution cryo-EM analysis of the *Salmonella* T3SS injectisome basal body. Nature. 2016;540(7634):597–601. https://doi.org/10.1038/nature20576.
Xian L, Yu G, Wei Y, Rufian JS, Li Y, Zhuang H, Xue H, Morcillo RJL, Macho AP. A bacterial effector protein hijacks plant metabolism to support pathogen nutrition. Cell Host Microbe. 2020;28(4):548-557.e7. https://doi.org/10.1016/j.chom.2020.07.003.
Xiao F, Mark Goodwin S, Xiao Y, Sun Z, Baker D, Tang X, Jenks MA, Zhou JM. *Arabidopsis* CYP86A2 represses *Pseudomonas syringae* type III genes and

is required for cuticle development. EMBO J. 2004;23:2903–13. https://doi.org/10.1038/sj.emboj.7600290.
Xiao Y, Lan L, Yin C, Deng X, Baker D, Zhou JM, Tang X. Two-component sensor RhpS promotes induction of *Pseudomonas syringae* type III secretion system by repressing negative regulator RhpR. Mol Plant Microbe Interact. 2007;20:223–34. https://doi.org/10.1094/mpmi-20-3-0223.
Xie Y, Shao X, Zhang Y, Liu J, Wang T, Zhang W, Hua C, Deng X. *Pseudomonas savastanoi* two-component system RhpRS switches between virulence and metabolism by tuning phosphorylation state and sensing nutritional conditions. Mbio. 2019;10(2):e02838-18. https://doi.org/10.1128/mbio.02838-18.
Xie T, Wu X, Luo L, Qu Y, Fan R, Wu S, Long Y, Zhao Z. Natural variation in the HrpL promoter renders the phytopathogen *Pseudomonas syringae* pv. *actinidiae* nonpathogenic. Mol Plant Pathol. 2023;24(3):262–71. https://doi.org/10.1111/mpp.13289.
Xin XF, Nomura K, Aung K, Velásquez AC, Yao J, Boutrot F, Chang JH, Zipfel C, He SY. Bacteria establish an aqueous living space in plants crucial for virulence. Nature. 2016;539:524–9. https://doi.org/10.1038/nature20166.
Xin XF, Kvitko B, He SY. *Pseudomonas syringae*: what it takes to be a pathogen. Nat Rev Microbiol. 2018;16:316–28. https://doi.org/10.1038/nrmicro.2018.17.
Xue Y, Zhu MJ. Unraveling enterohemorrhagic *Escherichia coli* infection: the promising role of dietary compounds and probiotics in bacterial elimination and host innate immunity boosting. Crit Rev Food Sci Nutr. 2023;63(11):1551–63.
Xue Y, Du M, Zhu MJ. Quercetin prevents escherichia coli O157:H7 adhesion to epithelial cells via suppressing focal adhesions. Front Microbiol. 2019;9:3278
Yan Q, Rogan CJ, Anderson JC. Development of a *Pseudomonas syringae*–*Arabidopsis* suspension cell infection system for investigating host metabolite-dependent regulation of type III secretion and pattern-triggered immunity. Mol Plant Microbe Interact. 2019;32:527–39. https://doi.org/10.1094/mpmi-10-18-0295-fi.
Yan Q, Rogan CJ, Pang YY, Davis EW, Anderson JC. Ancient co-option of an amino acid ABC transporter locus in *Pseudomonas syringae* for host signal-dependent virulence gene regulation. PLoS Path. 2020;16:e1008680. https://doi.org/10.1371/journal.ppat.1008680.
Yang S, Peng Q, San Francisco M, Wang Y, Zeng Q, Yang CH. Type III secretion system genes of *Dickeya dadantii* 3937 are induced by plant phenolic acids. PLoS ONE. 2008;3:e2973. https://doi.org/10.1371/journal.pone.0002973.
Yang L, Ding W, Xu Y, Wu D, Li S, Chen J, Guo B. New Insights into the antibacterial activity of hydroxycoumarins against *Ralstonia solanacearum*. Molecules. 2016;21(4):468. https://doi.org/10.3390/molecules21040468.
Yang L, Teixeira PJPL, Biswas S, Finkel OM, He Y, Salas-Gonzalez I, English ME, Epple P, Mieczkowski P, Dangl JL. *Pseudomonas syringae* type III effector HopBB1 promotes host transcriptional repressor degradation to regulate phytohormone responses and virulence. Cell Host Microbe. 2017;21:156–68. https://doi.org/10.1016/j.chom.2017.01.003.
Zarivach R, Vuckovic M, Deng W, Finlay BB, Strynadka NC. Structural analysis of a prototypical ATPase from the type III secretion system. Nat Struct Mol Biol. 2007;14(2):131–7. https://doi.org/10.1038/nsmb1196.
Zarivach R, Deng W, Vuckovic M, Felise HB, Nguyen HV, Miller SI, Finlay BB, Strynadka NC. Structural analysis of the essential self-cleaving type III secretion proteins EscU and SpaS. Nature. 2008;453(7191):124–127
Zhao F, Zhi T, Hu R, Fan R, Long Y, Tian F, Zhao Z. The OmpR-like transcription factor as a negative regulator of hrpR/S in *Pseudomonas syringae* pv. *actinidiae*. Int J Mol Sci. 2022;23(20):12306. https://doi.org/10.3390/ijms232012306.
Zheng X, Li X, Wang B, Cheng D, Li Y, Li W, Huang M, Tan X, Zhao G, Song B, Macho AP, Chen H, Xie C. A systematic screen of conserved *Ralstonia solanacearum* effectors reveals the role of RipAB, a nuclear-localized effector that suppresses immune responses in potato. Mol Plant Pathol. 2019;20(4):547–61. https://doi.org/10.1111/mpp.12774.
Zhi T, Liu Q, Xie T, Ding Y, Hu R, Sun Y, Fan R, Long Y, Zhao Z. Identification of genetic and chemical factors affecting type III secretion system expression in *Pseudomonas syringae* pv. *actinidiae* biovar 3 using a luciferase reporter construct. Phytopathology. 2022;112(8):1610–9. https://doi.org/10.1094/PHYTO-09-21-0404-R.
Zhou Z, Wu Y, Yang Y, Du M, Zhang X, Guo Y, Li C, Zhou JM. An *Arabidopsis* plasma membrane proton ATPase modulates JA signaling and is exploited by the *Pseudomonas syringae* effector protein AvrB for stomatal invasion. Plant Cell. 2015;27:2032–41. https://doi.org/10.1105/tpc.15.00466.
Zhou T, Yin C, Zhang Y, Shi H, Wang J, Sun L, Shao X, Gao R, Wang W, Deng X. Lon protease is involved in RhpRS-mediated regulation of type III secretion in *Pseudomonas syringae*. Mol Plant Microbe Interact. 2016;29:807–14. https://doi.org/10.1094/mpmi-06-16-0114-r.
Zwiesler-Vollick J, Plovanich-Jones AE, Nomura K, Bandyopadhyay S, Joardar V, Kunkel BN, He SY. Identification of novel hrp-regulated genes through functional genomic analysis of the *Pseudomonas syringae* pv. *tomato* DC3000 genome. Mol Microbiol. 2002;45:1207–18. https://doi.org/10.1046/j.1365-2958.2002.02964.x.