Mechanistic Insights Into Regulation of virulence and Diffusible Signal Factor (DSF)-mediated Quorum Sensing in *Xanthomonas citri*

Masters Essay

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Abstract

Xanthomonas citri subsp. *citri* (*Xcc*) is the etiological agent of a disease called citrus canker that affects almost all types of citrus crops. *Xcc* virulence factors are produced as a result of infection and they are controlled by a gene cluster *rpf* (regulation of pathogenicity factor), which encodes components of a cell-cell communication system called Quorum Sensing. Cell-to-cell signals of the Diffusible Signal Factor (DSF) family are cis-2-unsaturated fatty acids of differing chain length and branching pattern. The expression of virulence factors in plant pathogenic *Xanthomonas* spp. are regulated and coordinated by the system, diffusible signal factor (DSF)-mediated quorum sensing (QS). This regulation involves functions such as antibiotic tolerance, biofilm formation and the production of virulence factors. The virulence is influenced and regulated by certain factors such as adhesins, proteic structures for anchoring and protein secretion systems which involve the production of toxins and other substances. Perturbation of the DSF mediated QS can significantly reduce the severity of the bacterial disease and offers the possibility to control the infection. In this review, I have provided insights into the DSF-mediated QS regulation during plant-pathogen interactions in *Xcc*, the virulence factors affected by QS hampering, the main secretion systems that are active in virulence and their effect on the symptomatology of citrus canker. I have also shed light on the novel opportunities that this body of work has provided for disease control.

Keywords: *Xanthomonas citri*, DSF, quorum sensing, biofilm, *rpf,* adhesins, virulence, citrus canker, pathogenicity

1. *Introduction*

The genus Xanthomonas comprises a large group of plant pathogenic bacteria belonging to the group of Gamma proteobacteria. They are Gram-negative rod-shaped bacteria with a single polar flagellum whose colonies appear yellow due to a pigment named xanthomonadin and have a glossy appearance because of an exopolysaccharide (EPS) called xanthan [1]. Although bacteria from this genus can colonize a very wide array of hosts, its individual members are generally specialized to cause disease in a limited number of taxonomically related hosts. Numerous specialized virulence factors are employed by Xanthomonas bacteria to successfully invade the tissues of their susceptible hosts and multiply within them and cause disease. *Xanthomonas citri* subsp. *citri* (*Xcc*) is the etiological agent that causes citrus canker- a disease which generates substantial economic losses to the citrus industry worldwide either in terms of damage to trees (especially reduced fruit production), or the costs of its prevention and control. Lesions tend to appear on leaves, twigs and fruits which cause defoliation, premature fruit abscission and blemished fruit, and can eventually kill the entire plant. It is introduced to new areas through the movement of infected citrus fruits and seedlings, and unpremeditated re-introduction is highly likely despite the quarantine restrictions. Locally, *X. citri* is rapidly propagated by rainwater flowing over the surfaces of lesions and splashing onto uninfected shoots; spread is, therefore, greatest under conditions of high temperature, heavy rainfall and strong winds. Some areas of the world have eradicated citrus canker, others have on-going eradication programmes, however, this pathogen remains a threat to all citrus-growing regions [33].

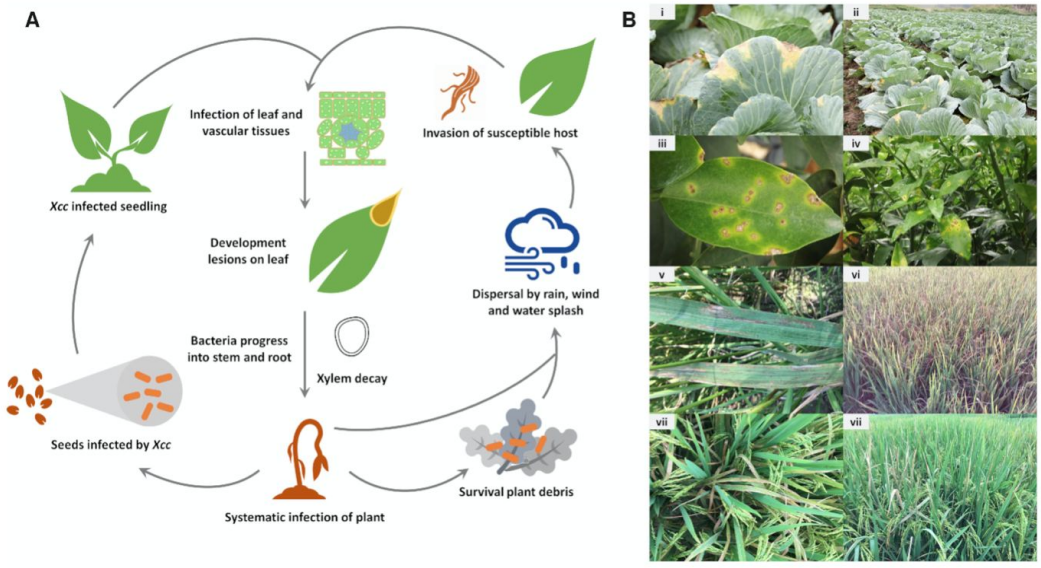

Figure 1. Life cycle and disease symptoms caused by *Xanthomonas citri* **(A)**. Natural openings of the leaves (the stomata) and wounds in the plant tissues are the sites of invasion and

colonization of the host. The characteristic canker lesion arises when the pathogen multiplies within the intercellular spaces, inducing cell hyperplasia thereby leading to rupture of the leaf epidermis and resulting in raised corky and spongy lesions surrounded by a water-soaked margin. Yellowish chlorotic rings are observed on leaves and fruits and when disease conditions develop better it produces general defoliation, tree decline, and premature fruit drop [6, 7] **(B)**. Images of disease symptoms caused by various *Xanthomonas* species. (i, ii) Black rot of cabbage caused by *Xanthomonas campestris* pv. *campestris*. (iii, iv) Citrus canker of citrus caused by *Xanthomonas citri* pv. *citri*. (v, vi) Bacterial leaf streak of rice caused by *Xanthomonas oryzae* pv. *oryzicola*. (vii, viii) Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (figure adapted from Shi-Qi An *et. al*. 2019).

Pathogenicity of microorganisms is due to the expression of virulence factors, which could be associated with their genetic, biochemical, or structural traits in the attempt to infect the host. Quorum sensing (QS) is the regulation of gene expression in response to fluctuations in cell-population density. It is a cell-cell communication system, which leads to the regulation of specific genes, and especially in bacteria control essential biological processes such as bioluminescence, antibiotic production, virulence, motility, and biofilm formation. *Xcc* harbours a wide range of virulence factors such as surface attachment structures, cell-wall-degrading enzymes, several secretion systems and their effectors, and a diffusible signal factor-mediated quorum sensing (DSF-mediated QS) system [3]. In *Xcc*, the production of particular pathogenicity factors is controlled by a cluster of genes called *rpf* (for regulation of pathogenicity factors), which encodes parts of a QS system mediated by molecules of the diffusible signal factor (DSF) [4]. Interference with cell-cell signalling, also termed quorum quenching, drastically decreases disease symptomatology and is a promising tool for biological control [5].

2. Factors affecting virulence in X. citri subsp. citri

2.1. Adhesins- Lipopolysaccharide and exopolysaccharide

The ability of the host to attach to the surface of the host cell is the fundamental step in bacterial colonization. Adhesins are bacterial surface structures anchored in its outer membrane that enables adhesion, which are mostly of polysaccharidic nature (lipopolysaccharides (LPS) and exopolysaccharides) but may also be of proteinaceous nature (type IV pili, chaperone/ usher pili, two-partner secretion) [6].

The lipopolysaccharides (LPS) form a crucial constituent of the outer membrane in Gram-negative bacteria and play multiple roles in plant-microbe interactions [7]. Antibacterial compounds are adverse environmental factors produced by the host cell and LPS acts as a barrier for protecting bacteria against them along with triggering/enhancing plant defence response. Therefore, LPS acts as a pathogen-associated molecular pattern (PAMP) identified in a wide variety of phytopathogenic bacteria and is sensed by animal as well as plant immune receptors .

Another bacterial polysaccharide released as an extracellular slime in the late stationary growth phase is the exopolysaccharide (EPS) in *Xanthomonas* known as xanthan gum. The intimate association of bacteria to abiotic and biotic surfaces is facilitated by EPS resulting in biofilm formation [8]. EPS in *xanthomonads* is composed by a backbone of b-1,4-linked d-glucose with trisaccharide side chains of mannose-(b-1, 4)-glucuronic acid(b-1,2)-mannose attached to alternate glucose residues in the backbone by 1,3 linkages [9]. Xanthan production in *Xanthomonas* is hierarchically directed by the *rpf* (regulation of pathogenicity factors) gene cluster [10].

2.2. Pigment xanthomonadin

Members of the group of *Xanthomonas* genus produce a yellow pigment termed xanthomonadin whose chemical structure is mono- or di-bromo-aryl polyene which binds to the outer membrane of the bacteria. A cluster of genes *pigA-pigG* are required for the biosynthesis of pigment and were first identified in *X. campestris* pv. *campestris* [11]. The xanthomonadin's main function is to prevent photobiological damage to the bacteria.

2.3. Proteic anchoring structures

Filamentous appendices known as Pili (fimbriae) are linked to the cell surface and their potential to induce hemagglutination, their anchoring site in bacteria (i.e., polar or omnidirectional) has been characterized. Various types of pili in bacteria of the *Xanthomonas* genus have been demonstrated or hypothesized to exist such as Type IV pili, chaperone/usher pili, and pili linked to different protein secretion systems [12].

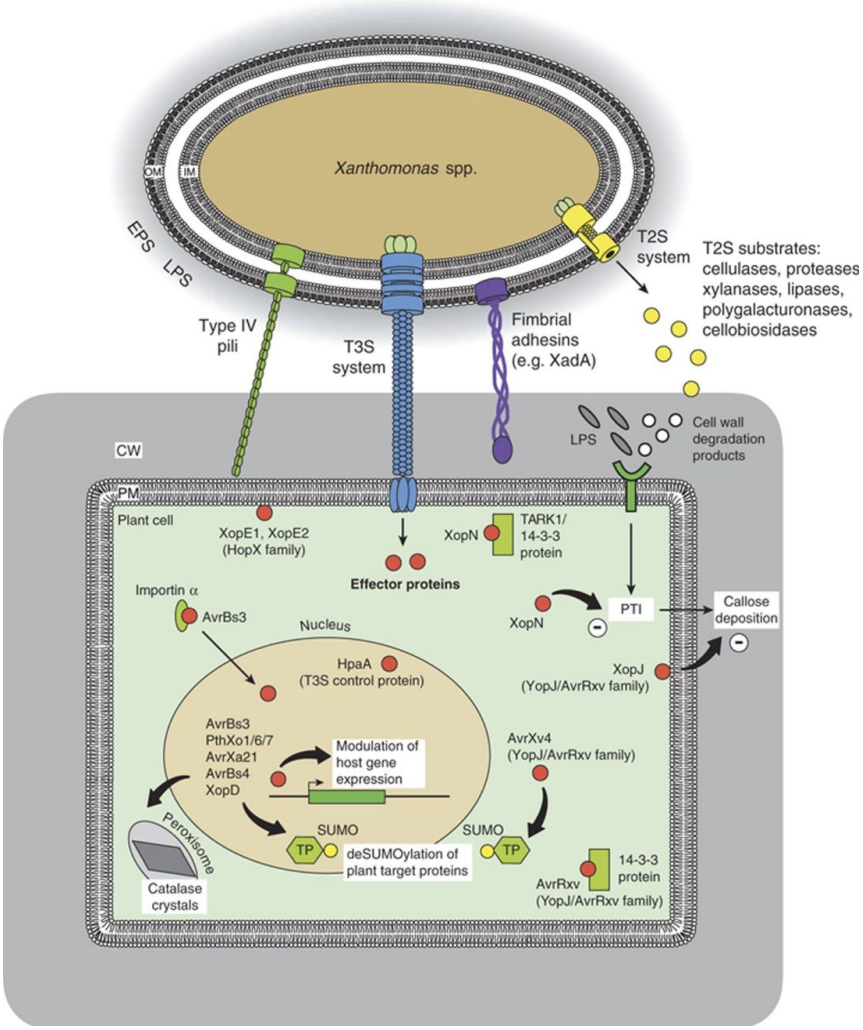

Figure 2. Model of known virulence factors from *Xanthomonas* spp. *Xanthomonas* spp. depend on T2S and T3S systems, adhesins, EPS and lipopolysaccharides (LPS) to successfully interact with their host plants [13].

3. *Protein secretion systems and their effectors influencing virulence in Xcc*

Xanthomonas spp. possess six types of protein secretion systems, type I to type VI, that differ significantly in their composition and function, and in the recognition of secretion substrates [14]. *Xcc* employs mainly T2 secretion system (T2SS), T3SS, T4SS, and T5SS and their effectors for invasion and multiplication in a susceptible host.

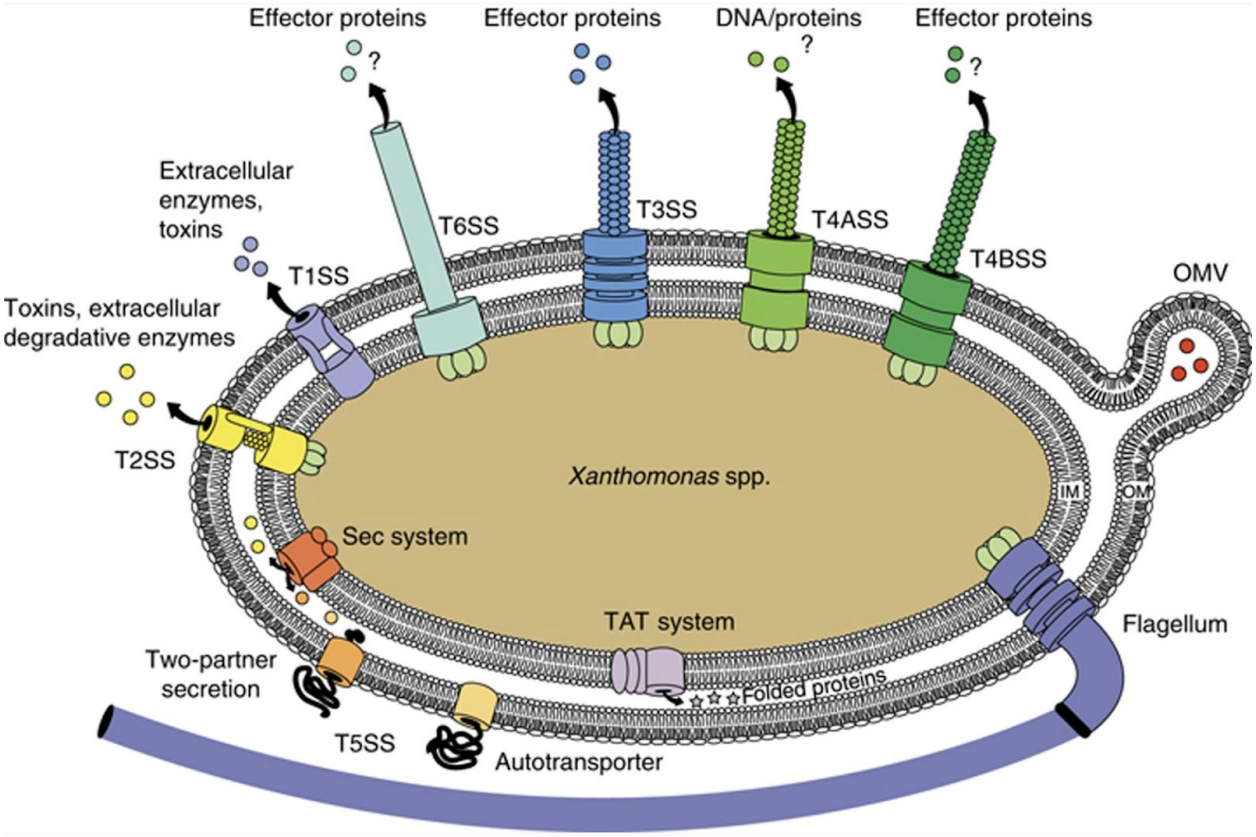

Figure 3. Diagrammatic representation of protein secretion systems from *Xanthomonas* spp. There are six types of protein secretion systems that are encoded- T1S to T6S. The T2S and T5S systems depend mainly on the Sec or the TAT system for protein transport across the inner membrane. T3S, T4S and T6S systems are associated with extracellular pilus structures and presumably translocate proteins into the host cell [13].

3.1. T2 and T3 secretion systems (T2SS, T3SS)

T2S system is the major protein secretion system mediating protein transport from the bacterial periplasm to the extracellular milieu. Toxins and extracellular enzymes such as proteases, lipases and cell wall-degrading enzymes are secreted that might contribute to the host-pathogen interaction. The T2S apparatus consists of 12–15 components, most of which are associated with the bacterial inner membrane [15]. A multimeric transmembrane channel in the outer membrane is formed by a member of the secretin protein family. Secretion across the outer membrane depends on a predicted periplasmic pilus that is continuously assembled and disassembled and thus T2S substrates are expelled out through the secretin channel [16]. The assembly of extracellular appendages of virulence-associated protein secretion systems such as T3S, T4S and T6S systems that are dedicated to effector protein translocation is facilitated by T2S substrates (figure 3).

The T3S system is essential for bacterial pathogenicity. In order to provide virulence factors (effectors) directly into host cells and consequently influence cell host activities, pathogenic bacteria employ the T3SS secretion system termed "needle" [12]. A *hrp* (hypersensitive response and pathogenicity) gene cluster is comprised of 26 genes from *hrpF* to *hpa2* encoding the T3SS proteins in *Xcc* [17]. Deletion mutant strains *hrpB, hrpB4, hrcV,* and *hrcN* in *Xcc* are known to completely eradicate the bacterial ability to cause citrus canker symptoms on susceptible citrus host [3], thereby validating the critical role of T3SS in virulence of *Xcc*.

There are 24 putative effectors identified in the *Xcc* genome and one of the principal effectors carried by the T3SS belongs to the family AvrBs3/PthA. These effectors contain functional domains are named TALE (Transcription Activators Like Effector) which are characteristic of eukaryotic transcriptional activator [36]. TALE contains a central repeat domain that identifies the host DNA in a highly specific manner. Each repeating unit contains 34 amino acids, with 12 and 13 hyper-variable amino acids termed VRD (Variable Repeat Di-residue). Tremendous capacity is provided to TALEs (due to composition and arrangement of VRDs) to identify DNA host, and binding occurs with a high degree of specificity to a particular region at the promoter of target gene known as EBE (Effector Binding Element). Xcc contains four genes (PthA) that encode TALE, of which PthA4 is known to be essential for the formation of citrus canker lesions. The TALE in Xcc encoded by PthA4 gene induces a gene in the susceptible host resulting in the formation of erumpent lesions [38].

Molecular recognition events eliciting a virulence response show that the PthA protein is secreted into the host cells through the expression of the *pthA* gene in the pathogen through a functional type III secretion system. The PthA protein mobilizes to the nucleus of host cells and combines with the DNA of the host cells. Division, enlargement and death of host cells occurs due to the transcription activation of host cells. One of the PthA homologues, Ap11 (Avir/PthA-like), is thought to be a signal specific for canker formation [2].

4. Control of virulence gene expression by Quorum Sensing

The infection cycle of *Xanthomonas* can be divided into the epiphytic stage and the endophytic stage. The epiphytic stage initiates once bacteria are introduced into the aerial tissues of a new host, usually leaf or fruit tissue and continues until the entrance into the host tissue via the plant natural openings and wounds. Once inside a host plant, the bacteria enter the endophytic stage and colonise the host. In order to enable bacteria to adapt to environmental changes, to colonize new habitats, resist host defence and antibiotic action, strengthen competitiveness, and take advantage of new food sources the development of an intercellular communication system is a standard characteristic [55]. Quorum sensing (QS) is a system in which implementation and recognition of diffusible signal molecules influence cooperative multicellular behaviour. QS is a signalling mechanism for coordinating the expression of genes at the population level. The process of QS relies upon the production, release, and detection of small signalling molecules known as auto-inducers (AIs). Bacteria within the genus *Xanthomonas* encode a cell-cell signalling or QS system which uses molecules from the diffusible signal factor (DSF) family as AI.

In *X. campestris* pv. *campestris*, the QS signal (DSF) *cis*-11-methyl-2-dodecenoic acid was identified and shown to regulate the expression of at least 165 genes including putative virulence genes [4, 18, 19, 20, 21]. DSF is synthesised by putative enoyl-CoA hydratase RpfF and the fatty acyl-CoA ligase RpfB, both encoded by the *rpf* (regulation of pathogenicity factor) gene cluster including rpfB, rpfF and rpfGHC [22].
RpfB is a fatty Acyl-CoA ligase involved in the turnover of the DSF family of signals in *Xanthomonas* [39]. The RpfF protein, functioning as a putative enoyl-CoA hydratase, is responsible for the synthesis of DSF. RpfC and RpfG consist of a two-component system involved in DSF perception and signal transduction. DSF is assumed to be able to diffuse across the bacterial membranes due to its lipophilic nature and accumulates in the early stationary growth phase in the external milieu [23]. A two-component signal transduction system consisting of the sensor kinase RpfC and the response regulator RpfG presumably help in sensing DSF [24] (Fig. 4). Mutations in *rpfF*, *rpfG* or *rpfC*, result in decreased production of EPS, extracellular enzymes and altered biofilm formation in certain media, suggesting that DSF signaling is involved in the regulation of virulence factors.

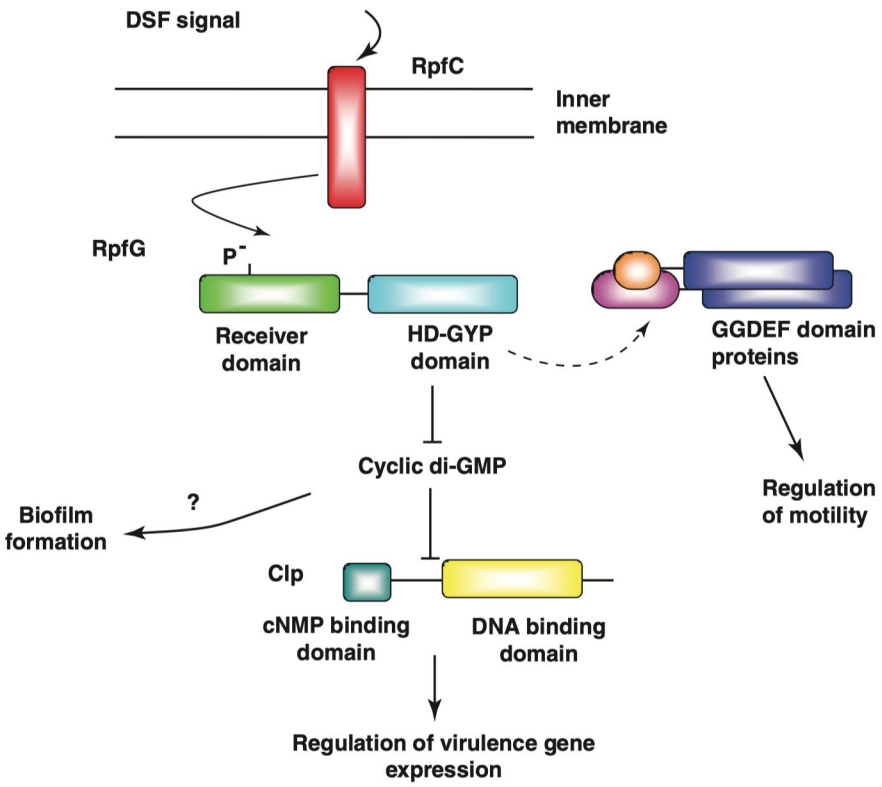

Figure 4. Schematic representation of expression of different DSF-regulated functions in Xcc obtained via Multiple signal transduction pathways (figure adapted from Ryan R P *et. al*. 2011 [25]).

Perception of DSF by RpfC leads the phosphorylation of RpfG, it triggers the activation of RpfG as a cyclic di-GMP phosphodiesterase reducing the level of cyclic di-GMP and releasing Clp that promotes the synthesis of extracellular enzymes and EPS. Autophosphorylation and phosphotransfer to the REC domain of RpfG are achieved by the perception of DSF. An activity associated with the HD-GYP domain i.e., activation of RpfG as a cyclic di-GMP phosphodiesterase occurs as a result (refer figure 4). Synthesis of extracellular enzymes and EPS is promoted by consequent reduction of cyclic di-GMP levels but leads to inhibition of biofilm formation. DSF signalling also promotes RpfG binding to two proteins with a GGDEF diguanylate cyclase domain. This physical interaction has no influence on the biosynthesis of extracellular enzymes and EPS or on biofilm formation acts to control motility. EPS involves the transcriptional activator Clp and Cyclic di-GMP effects on the synthesis of extracellular enzymes. Cyclic di-GMP binds to Clp and prevents binding of Clp to the promoters of target genes that include those encoding extracellular enzymes and EPS biosynthesis. The reduction in cyclic di-GMP levels as a consequence of DSF perception allows Clp to bind to DNA and

activate gene expression. The negative regulation of biofilm formation by the Rpf/DSF system is exerted independently of Clp and could involve control of expression of the xagABC gene cluster which encodes putative glycosyl transferases required for biofilm formation. Expression of the xagABC gene cluster, an effect that requires the two-component RpfC–RpfG system is negatively influenced by DSF. It has been speculated that in the absence of DSF i.e. conditions in which biofilm formation occurs, the expression of xagABC (and other functions) required for biofilm formation is positively influenced by the high intracellular levels of cyclic di-GMP [25].

RpfC is a complex kinase sensor positioned in the cytoplasmic membrane, which is associated with a cytoplasmic regulator is involved in DSF detection and transduction in bacteria. The best studied of these systems is RpfCF/RpfG of *X. campestris*, which is shared by all *Xanthomonas* species [25]. Being a complex sensor kinase, RpfC is composed of (i) a histidine kinase domain (HisKA) coupled to an ATPase, (ii) a sensory domain, which consists of five transmembrane helices with periplasmic and endoplasmic loops, that has the function of sensing DSF levels, (iii) a CheY-like two components domain (REC), and (iv) histidine phosphotransferase domain (HPT). The regulator RpfG contains (i) a REC domain and (ii) a HD-GYP domain, which is a phosphodiesterase and is involved in the degradation of the second messenger cyclic di-GMP (figure 4).

Phosphorylation of RpfG activates the cyclic di-GMP phosphodiesterase, which results in modifications in the level of cyclic di-GMP in the cell. It is known that RpfG, the regulator of DSF signalling cascade, has a Che-Y like receiver domain (REC) that is connected to a HD-GYP domain, and has phosphor di esterase activity, which takes part in the degradation of the second messenger cyclic di-GMP. As outlined above, changes in intracellular second messenger cyclic di-GMP trigger major changes in bacterial phenotype which are consequently brought about by the phosphorylation of HD-GYP domain in the RpfG regulator [26]. This affects the synthesis of virulence factors such as extracellular enzymes, EPS, biofilm dispersal, and motility [24]. The cyclic di-GMP influences the global transcriptional activator Clp (cAMP receptor-like protein) in terms of the effect of RpfG in the synthesis of extracellular enzymes and biofilm formation [37].

5. Signal interference and infection control by Quorum Quenching

QS (intercellular communication system) enables bacteria to coordinate the expression of virulence genes and colonize new habitats in order to establish and develop pathogenicity. In general, inhibition of quorum sensing can lead to certain phenotypic alterations, such as virulence reduction, reduced biofilm formation, and increased bacterial sensitivity to treatments. Interruption of QS in X. citri subsp. citri leads to a down-regulation of genes that encode adhesins and fimbria, hence disturbing biofilm formation and epiphytic fitness which are factors that are quintessential in the early stages of pathogenicity. It is essential to alter the balance between the host defence mechanisms and the pathogen virulence in favour of the host and any molecule that could help achieve this might be of practical value to keep the infection under control. Interfering with QS could involve signal degradation (quorum quenching) or signal overproduction (pathogen confusion) [31, 32].

Sequence alignment and structural analysis of the *Xanthomonas* spp. enabled the identification of two putative catalytic glutamate residues (Glu141 and Glu161), which are preserved in enoyl-CoA hydratase/dehydratase. It was demonstrated that the substitution of these two residues in RpfF completely eradicated the DSF production, emphasizing its critical role in DSF biosynthesis [27]. This was achieved by directly studying the DSF mechanism in *Xanthomonas*.

5.1. Generation of the rpfF, rpfC, and rpfG mutants of X. citri subsp. citri.

Deletion mutants of the three critical genes involved in DSF family signal synthesis, detection, and transduction—*rpfF, rpfC, and rpfG*—were generated using double cross-over recombination to investigate the role of DSF family signal-mediated QS in citrus canker infection. To mimic the natural infection process of *X. citri* subsp. *citri*, pathogenicity assay was conducted in Duncan grapefruit leaves which were inoculated with bacterial suspensions by spraying on the abaxial surface. The inoculated plants were subject to 100% relative humidity and then kept in a greenhouse (approximately 50% relative humidity) for the symptoms to develop. Mutations in rpfF, rpfC, or rpfG reduced the attachment of *Xanthomonas citri* subsp. *citri* to abiotic and biotic surfaces [40].

Similarly, *in vitro* assay was performed where the *rpfF, rpfC, and rpfG* mutants could not form compact pellets, unlike the wild-type strain, when centrifuged from the liquid

medium nutrient broth (NB). These mutants were assayed for motility and extracellular protease production, which are well-known phenotypes controlled by DSF-mediated QS to confirm the mutations in the QS pathway, [4, 25]. A significant decrease was observed by the *rpfF, rpfC, and rpfG* mutants in the production of proteases and had decreased motility. Basically, the conservation of *rpfF, rpfG*, and *rpfC* genes indicated the conservation of a DSF-mediated signalling system.

5.2. Mutations in wxacO and rfbC genes to alter biofilm formation

The *wxacO* gene is predicted to encode a putative transmembrane protein and *rfbC* to encode a truncated O-antigen biosynthesis protein. Both wxacO and rfbC are involved in the biosynthesis of LPS. It was predicted via sequence analyses by Popot *et. al* that the wxacO product indicated that this gene is a member of a transmembrane protein family, which contains diverse enzymes or components involved in the assembly and/or export of surface polysaccharides [28], and the rfbC gene is a part of the GT2 subfamily containing glycosyl transferase for the biosynthesis of polysaccharides [29]. The *wxacO* and *rfbC* mutants have shown reduced swimming motility compared with the wild-type strain, in line with previous reports for LPS mutants of other bacteria. This could mean that the defect in swimming motility of LPS mutants could be due to loss of formation or function of flagella. Therefore, it can be emphasized that a structurally intact LPS is important for biofilm formation and bacterial ecological competence, as well as virulence, of the citrus canker pathogen and the absence of genes wxacO and rfbC hamper the virulence of *Xcc* [30].

6. Conclusion and Future Perspectives

In the past decade and as stated above, a large number of publications revealed the complexity of virulence factors employed by *Xanthomonas* spp. to conquer their respective host plants that include valuable crop species worldwide. The bacterial ability to adhere to and to communicate with host cells is very crucial for successful infections. Bacterial surface structures and secreted proteins are most known virulence factors that are likely to promote nutrient procurement by the bacterium and suppress plant defence responses. Bacteria also translocate effector proteins into the plant cell cytosol for improved manipulation of plant cells. One of the key events during the host-pathogen interaction is the effector protein translocation by the T3 system and has therefore been studied intensively. However, presumably due to functional redundancies among secreted proteins, the functional characterization of effector proteins and other virulence factors is

often complicated by the fact that individual deletion mutants are not impaired in virulence. One of the foremost focuses of future research is the extensive analysis of molecular mechanisms behind the activity of secreted virulence factors. Therefore, it is believed that the detailed characterization of effector proteins will not only shed light on bacterial virulence strategies but also provide clues about plant developmental processes [13].

In this review, we focus on the differences in candidate genes and factors associated with the pathogenicity and host range of *Xanthomonas citri* subsp. *citri.* When taking into consideration the similarity of most of the known pathogenic factors and genes of *Xcc*, it seems that, in addition to strain-specific genes that are likely to be the major driving force for strain differentiation, there are other unknown factors and genes that require further study. In the case of DSF-mediated QS, the perception of DSF in a RpfC Xcc is fixed to the phosphorylation of HD-GYP domain in the RpfG regulator and the resulting changes in intracellular second messenger cyclic di-GMP that trigger significant changes in the bacterial phenotype [26]. DSF/Rpf -mediated QS regulation from both pathogen and host sides during the biotrophic interactions between *Xcc* and citrus is analyzed in-depth. A model describes the major molecular and physiological aspects regulated by the DSF/Rpf- mediated QS during early stages of infection (Fig. 4). These findings support the hypothesis that the DSF/Rpf- mediated QS in *Xcc* accentuates diverse pathogenesis traits to develop and promote bacterial adaptation to the host milieu, and triggers several changes in plant immunity and physiology furthering the pathogen for successful infection. This affords novel insights into the function of the DSF/Rpf- mediated QS regulatory system in the pathogenic interactions between *Xanthomonas* and its host plants and helps grow our current perception of DSF-mediated QS regulation and thereby adds to our comprehensive understanding of plant-pathogen interactions.

For DSF family signal-mediated QS in *X. citri* subsp. *citri*, the RpfC-RpfG two-component system is a principal and conserved signal perception and transduction system. The complexity of the QS pathway and the involvement of additional sensory mechanisms in *X. citri* subsp. *citri*. are indicated by the unique genes controlled by RpfF alone. It is presumed that the unique genes controlled by RpfC and RpfG, promote the likelihood that RpfC and RpfG play more ubiquitous roles in gene regulation other than transduction of DSF signals. More attention on interference with DSF signalling could afford a route to the control of the disease.

For the understanding of mechanism and factors involved in biofilm formation, a hypothetical gene wxacO, as well as the rfbC gene, of *Xcc* strain 306 were characterized.

The disruption of wxacO or rfbC affected the virulence of *Xcc* strain 306 on host leaves. The outcome was that LPS-defective mutants of the pathogens showed reduced virulence compared to the corresponding wild-type strain. These mutations hampered biofilm formation and reduced the motility of the bacteria thereby leading to a reduction in the spread of canker. This explains that both wxacO and rfbC are involved in the biosynthesis of LPS, and a structurally intact LPS is important for biofilm formation and bacterial ecological competence, as well as virulence, of the citrus canker pathogen.

Numerous enzymatic and non-enzymatic signal interference mechanisms that quench microbial QS signalling were identified by the discovery of microbial QS signals and the signalling mechanisms. There is evidence that such signal interference mechanisms can be developed as affirming approaches to control microbial infection and biofilm formation. These mechanisms exist in the host organisms of bacterial pathogens and not only in microorganisms thereby, highlighting their potential implications in microbial ecology and in host-pathogen interactions. Investigation of QS and signal interference mechanisms might significantly broaden the scope of research in microbiology.

In conclusion, the aim of this review was not only to provide a summary of our current knowledge of virulence factors from *Xanthomonas citri* subsp. *citri* but also to show that we are just beginning to understand bacterial virulence strategies by analyzing their protein secretion systems, cell-cell communication systems, diverse signalling mechanisms and strategies for interference, with consequences for disease control.

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