Chapter

Xanthomonas citri ssp. *citri* Pathogenicity, a Review

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Abstract

The infectious process of plant by bacteria is not a simple, isolated and fortuitous event. Instead, it requires a vast collection of molecular and cell singularities present in bacteria in order to reach target tissues and ensure successful cell thriving. The bacterium *Xanthomonas citri* ssp. *citri* is the etiological agent of citrus canker, this disease affects almost all types of commercial citrus crops. In this chapter we review the main structural and functional bacterial features at phenotypical and genotypical level that are responsible for the symptomatology and disease spread in a susceptible host. Biological features such as: bacterial attachment, antagonism, effector production, quorum sensing regulation and genetic plasticity are the main topics of this review.

Keywords: Biofilm, Secondary Metabolites, Antibiotic, Xanthomonadine, Quorum sensing

1. Introduction

The surface of the plants is one of the most hostile environments, prevailing factors at the phyllosphere such as: the low availability of nutrients, the high incidence of UV rays, the fluctuating periods of temperature and humidity, mechanical disruption by winds, antibacterial compounds produced by the host plant or by microorganisms member of leaf microbiome, among others, make the bacterial persistence and survival itself a pathogenicity strategy. Due the symptoms development ceases when one pathway involved in the bacterial epiphytic survival is seriously threatened [1]. In phytopathogenic bacteria whose infection route is the phyllosphere, it is important to understand how phenotypic traits upset to ensure survival and surface fitness, and how these traits interact with the phyllosphere microbiome in order to secure the onset of infection (**Figure 1**). Besides, over the time, on a large-scale, plant leaves will age and fall, thus, the phyllosphere bacteria must have to anticipate living outside of the leaf, for example in the air, soil or reach to young leaves [2].

Bacterium *Xanthomonas citri* ssp. *citri* (*Xcc*) is the etiological agent of bacterial citrus canker. This bacterium is equipped with a huge arsenal of cellular structures that allow its survival in the phyllosphere before it reaches the target mesophyll tissue. *Xcc* secretes toxins that directly affect the survival of its competitors. Once in the mesophilic tissue *Xcc* produces effectors that are responsible by the appearance of spongy and corky pathognomonic lesion of citrus canker. In this chapter we will review the both bacterial life style outside and inside of the host.

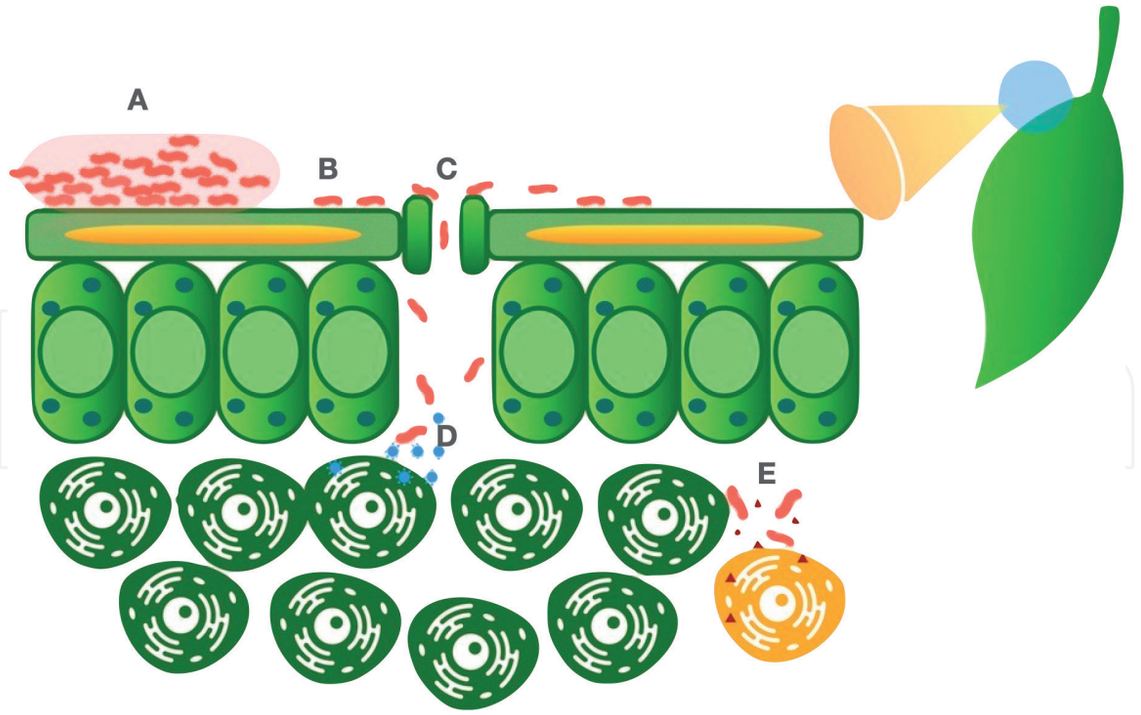

Figure 1.
Phytopathogenic bacteria plant infection. ***A.*** *Surface leaf survivor and biofilm formation.* ***B.*** *Bacterial movement to natural opening on leaf.* ***C.*** *Phytotoxins secretion to modulate stomatal closure.* ***D.*** *Effector secretion that affect the cell host behavior.* ***E.*** *Degrading cell wall plant protein secretion.*

2. *Xanthomonas citri* ssp. *citri* taxonomy

The bacterium *Xantomonas citri* ssp. *citri* is a gram negative rod shape bacteria with a single polar flagellum. *Xcc* belongs to the *Xanthomonas* genus from the gamma proteobacteria group. This genus is constituted by 28 species and more than 150 pathovars [3]. In the early 1900s, due to pathogenicity experiments, the bacterium was classified as *Pseudomonas citri* [4]. Subsequently, the bacterium was classified into different genus such as: *phytomonas bacterium* and finally at late 1930 classified as *Xanthomonas citri* [5]. The bacterium continued in *X. citri* until 1978, when it was classified in *X. campestris* pv. *citri* in order to reserve citri at the specific level [6]. In 1989 Gabriel suggest the replaced of bacterium as *X. citri* [7]. Using DNA–DNA hybridization approach and based on renaturation rates, the bacterium was classified as *X. axonopodis* pv. *citri* by Vauterin [8]. Lately, It was suggested major changes to Xanthomond taxonomy, it which were based on multilocus sequence analysis (MLSA) and digital DNA–DNA hybridization of whole genome nucleotide, the author has been recommend the names *Xanthomonas citri ssp citri* for the etiological agent of citrus cancer type A [9].

3. Microbe- host interaction

3.1 Microbe -host interaction outside the susceptible host "epiphytic life style"

Bacterial citrus canker disease cycle begins with the deposit of inoculum of *XCC* at the leaf surface by rain splash. Subsequently, the bacteria move toward the natural opening of leaves, the stomata, then, the bacteria reach the apoplastic space and start the infection process inside the host "endophytic lifestyle". In this section we are going to focus on the structures, toxins, molecules and extracellular substances that favor and promote the epiphytic interaction between XCC and susceptible citrus host.

3.1.1 Type IV pili

Several bacterial genera are endowed with filamentous appendages called pili. These filamentous organelles include the chaperon- Usher pili, type IV pili (T4P) and gram-positive pili. All types of pili are homopolymers ensembled of thousands of units of pilin protein. The outstanding function of pili is the attachment to surfaces, besides, in *Xcc* pili type IV is also responsible for the twitching motility and biofilm formation [10]. Type 4 pili is unique in its dynamism, since, it polymerizes and depolymerizes in very fast cycles, which leads to instantaneous extension and retraction cycles producing considerable mechanical force [11], as a consequence, this organelle could attract several substrates like DNA or bacteriophages in order to internalize to periplasmic space, as well as to secrete protein across the membrane [12]. Twitching motility is a bacterial displacement that able to cell to move over humid on organic and inorganic surfaces on a fashion independent of flagella [13]. In the process of biofilm development, the T4P contributes in the initial steps exactly in the reversible attachment phase and subsequently, in the formation of mushroom microcolonies. Contribution of T4P in the pathogenicity in XCC is not completely demonstrated, however, the mutation of *pilM* gene responsible to encode a membrane protein that participate in the T4P pili ensemble reduce drastically the bacterial virulence [10].

3.1.2 Type V secretion system (non fimbrial adhesins)

Xanthomonads encode type V secretion system (T5SS), it which has a function as non fimbrial adhesins [14]. Compared with the other bacterial secretion systems, the secretion system 5 is one of the simplest complexities from the structural point of view; it is smaller and has only presence at the outer membrane of gram negative bacteria [15]. This T5SS do not have a direct energy source, there is no ATP accessible in the periplasm space neither proton gradient. Consequently, the name of autotransporter has been coined for the this T5SS [16]. The T5SS is comprised of two domains: the β barrel that is located at the out membrane and a secreted passenger. There are five subtypes of T5SS from Va-Ve and recently a new subtype the Vf has been discovered [17]. The bacterium *Xcc* is endowed with three subclasses of T5SS: Va, Vb and Vc. (**Figure 2**). Va is a classical auto-transporter, it which transport proteases, lipases and adhesins. The type Vb is a secretion system knowing as Two-Partner Secretion System (TPS), which is composed by a translocator protein and a cognate passenger protein. Translocation from the cytoplasm to the periplasm space occurs by Sec translocase pathway once the perception of amino terminal from signaling peptide is done. The passenger protein has effector function and is termed TpsA. It is transported by TpsB, which forms a pore in the outer membrane in order to enable the TpsA translocation. TpsB also comprise two periplasmic domains. TpsB typically contains a 16-stranded beta-barrel domain that forms the outer membrane pore and two periplasmic POTRA (Polypeptide transport associated). Its function is the recognition of the cognate partner via binding to a TPS domain in TpsA.

The T5SS subclass Vc have a trimeric transporter adhesin conformation, this surface exposed adhesin assembles as homotrimeric structure at the outer membrane [18]. Proteomic and functional studies involving T5SS have revealed roles in pathogenicity to host primarily implicated in the adhesion, especially in the initial steps of pathogenicity process [19, 20].

3.1.3 Xanthomonadin pigment

Xanthomonads bacteria produce a yellow pigment membrane bound known as Xanthomonadins. Several studies have shown that Xanthomonadin has a pivotal

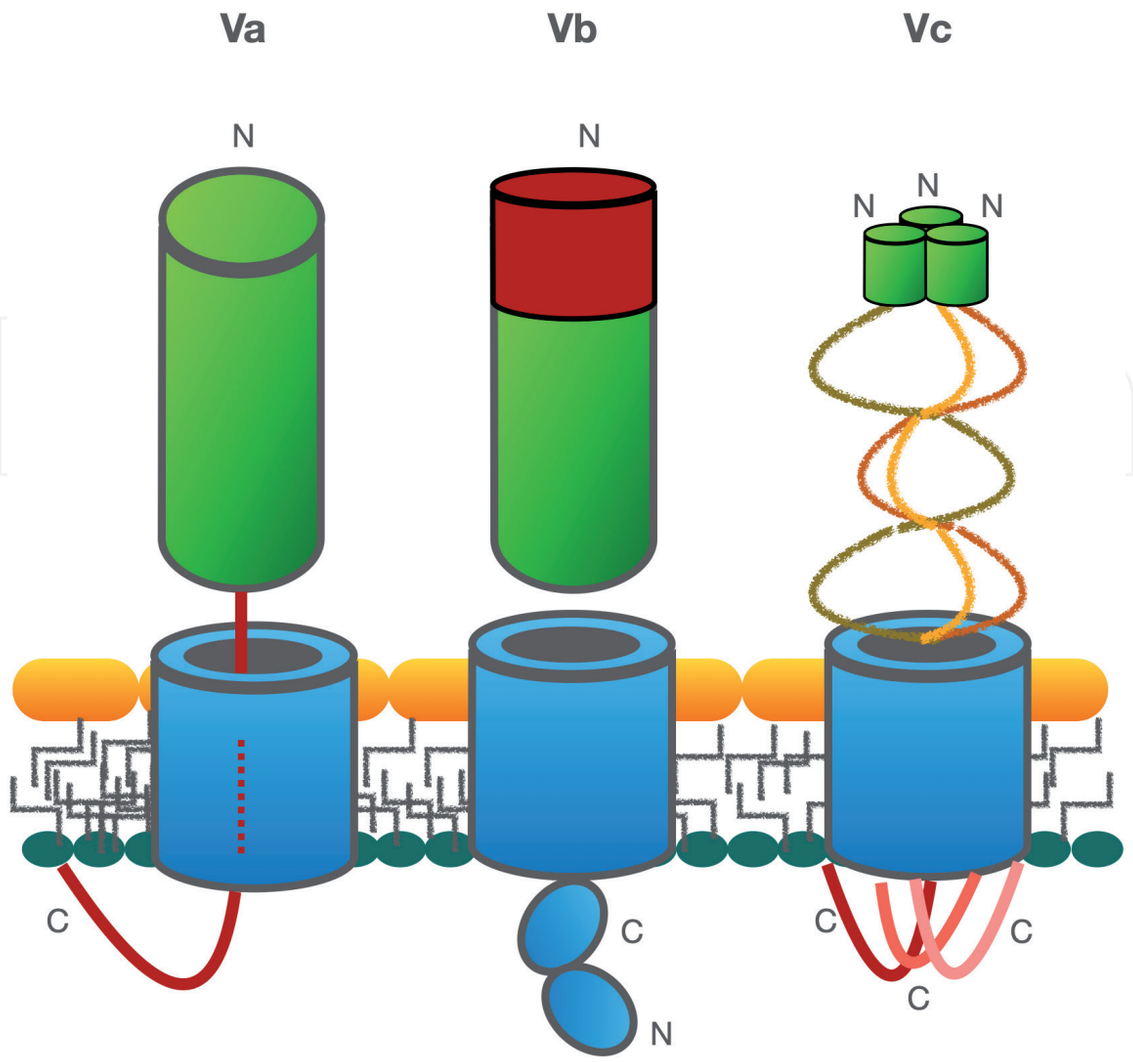

Figure 2.
Schematic representation of T5SS present in Xcc. β barrel domain and POTRA are characterized with blue, linker, passengers transported are represented in green and two partner secretion system domain are characterized with red.

role in a epiphytic survival and in plant-pathogen interaction [21, 22]. In the early years this yellow pigment was associated with the carotenoids. However, it was only until its full characterization was achieved that this pigment represents a unique group of aryl-polyene, water insoluble new type of pigment [23]. Genomic analysis shows that a region near to 25.4 kb contains seven transcriptional units (*pigA*, *pigB, pigC, pigD, pigE, pigf* and *pigG).* This gene cluster encodes necessary elements for Xanthomonadin biosynthesis [24]. Biological roles of xanthomonadin in a pathogenicity context are: (i). *favor the bacterial epiphytic survival,* since, Xanthomonadin avoid the photodamage produced by UV light irradiation that results in ROS production. Similar as structural related carotenoids, Xanthomonadin absorbs wavelengths between UV-C to red light. This pigment gives the bacteria additional advantages against the other phyllosphere colonizer bacteria as it is to deal with stress related factors such as UV irradiation and consequently the photo oxidative damage. Xanthomonadin also offers protection against visible light in the presence of exogenous photosensitizers. Cellular location of Xanthomadin (outer membrane) strongly suggests that this pigment stabilizes cell membrane in the epiphytic phase of this phytopathogenic bacterium. Previous studies in which *Xcc* deletion mutants of the *pig* genes were used and which were inoculated using the needleless syringe pressure technique did not show a significant reduction in virulence compared to the wild type phenotype inoculated using the same technique. Instead, when using the spray

infection method, that resembles the natural infection method, it which involve the epiphytic fitness stage, the *Xcc* pig mutant strains display great reduction in the virulence compare with the wild type phenotypes [25]. (ii) *Antioxidant activity*, the oxidative stressors as ROS and H_20_2 injury the membranes, DNA and proteins, the carotenoids pigments could efficiently quench the ROS.

3.1.4 EPS xanthan and LPS

The EPS in Xanthomonas is named as xanthan, this polysaccharide surrounds the outer membrane through non-covalent ligations [26]. Pathogenicity roles in Xanthomonas genus differ greatly depending on specie, e.g. in *Xanthomonas campestris*, xanthan suppresses induced innate immunity by calcium chelation [27]. In addition xanthan increases the plant susceptibility to *X. campestris* due to avoiding the callose deposition [28]. In Xac there is controversy regarding the direct participation of xanthan in the pathogenicity process, while some authors find just a discrete participation in the epiphytic survival [29], another study shows that xanthan deletion mutants reduce the surface leave colonization ability and consequently the severity of citrus canker disease was deeply reduced [30]. Xanthan is a key component in the biofilm formation. The gene cluster *gum* is responsible for the xanthan production and exportation. This gene cluster comprise 12 successive genes with one operon-like identical direction of transcription i.e. *gumB* to *gumM.* The first two genes of cluster *gumB* and *gumC* encode components of channel than spans the outmembrane and the periplasmic space and enable the xanthan secretion [31].

The LPS is the major component of the outer leaflet of the outer membrane. The LPS in *Xcc* have a classic conformation being a tripartite glycoconjugate forming by: lipid A that carries a core oligosaccharide and polysaccharide the O- antigen. LPS that lack the O-antigen are named as lipooligosaccharide (LOS) or rough-type LPS. LPS has an essential role in bacterial growth acting as a barrier for antibacterial compounds and delivering protection against stress as well contributing to the structural proprieties of outer membrane. Lipid A is fairly conserved in most gram-negative bacteria, however, in Xanthomonas genus there is variation in the core oligosaccharide and O antigen structures, there may even be variation between the different species of Xanthomonas [32]. Nowadays is has been established that LPS has a double role in plant-microbial interaction; (i) elicitor of immunity plant response and (ii) It has a role in the promotion of virulence, because it acts as a barrier against antimicrobial activity compounds produced by root hair. *Xcc* is able to overwhelming plant defense responses induced by LPS.

3.1.5 Quorum sensing and biofilm formation

One discovery in microbiology that completely changed the conception of microbial ecology in the last two decades was the establishment of cooperative behavior in bacterial populations. This social behavior allows members of the bacterial community to adapt to new ecological niches, colonize new habitats, gain a competitive advantage against potential competitors and resist or avoid the host defense [33]. This cooperative behavior is based on a cell to cell communication system known as Quorum Sensing. Quorum sensing (QS) is a system of bacterial cell–cell communication that enables the microorganism to sense a minimum number of cells (quorum) in order to respond to external stimuli in a concerted fashion [34]. The process of QS relies upon the production, release and detection of small signaling molecules called auto-inducers. Each bacterial cell produces a basal quantity of auto-inducers, which are exported to the extracellular environment and

reflect bacterial population density. At high cell densities, the auto-inducers reach a critical concentration, at which point they are recognized by their cognate receptor, triggering a cascade of biological functions [35].

The autoinducer in *Xcc* is a short chain fatty acid molecule known as DSF (Diffusible Signal Factor). Once this DSF accumulates at the extracellular space up to a critical level, it is sensed by its cognate receptor and triggers a cascade of biological function via the internal second messenger cyclic di-GMP, which is involved in virulence, resistance and biofilm formation. The encoding genes for quorum sensing components in *Xcc* form a cluster termed as *rpf* (Regulation of Pathogenicity Factors). For detailed revision of DSF quorum sensing circuit in *Xcc* [36].

Once *Xcc* reaches a leaf surface, it begins the initial adhesion process that was mention above. This attachment is followed by the formation of biofilm-like structures. Biofilm classical definition is an aggregated composed by several bacterial communities, which are embedded in a self-produced matrix of EPS, these bacterial cells are attached to each other or/and to a surface [37]. Biofilm is composed by polysaccharides, nucleic acids (eDNA), proteins, and have a pivotal role in attachment and protection against biotic and abiotic factors. In *Xcc* the biofilm formation in leaf and fruit surfaces is a main virulence factor in the early stage of development of citrus canker disease. In *Xcc* biofilm formation and dispersion is modulated by the quorum sensing autoinducer molecule DSF. How it was mention before DSF autoinducer promotes the biofilm formation because it stimulates the EPS production and pilus ensemble. On the other hand, DSF negatively regulates the biofilm formation because; it upregulates β 1–4 mannanase, ManA, leading to EPS dispersion and disassembly of biofilm [38]. Our previous study shown that quorum sensing signaling plays an essential role in the epiphytic stage survival, which is crucial at the early phase of pathogenicity development. Since, quorum quenchers bacteria belonging to genus Pseudomonas and Bacillus, it which were isolated from leaves of susceptible citrus host, which displayed the ability to disrupt the DSF pathway in *Xcc* and reduce citrus canker severity in a high susceptible citrus host [34].

3.1.6 T4SS and T6SS potentiates the Xcc antagonism with bacteria inhabiting the phylloplane and the soil amoeba

Nutrient limitation in the phyllosphere additional to environmental changes conditions, make the surface of the leaves one of the most hostile, restrictive and competitive habitats [38]. The type IV protein secretion system is used by bacteria to inject proteins and/or DNA into the prokaryotes and eukaryotes targets. Xanthomonas are endowed with genes that encode components of T4SS, the encoding genes VirB7, VirB8 and VirB9 responsible for the outer membrane pore formation. Genes that encode for VirB3, VirB4, VirB6, VirB8, Vir11, VirD4 and VirB10, responsible for the pore formation at the inner membrane. Finally, the gene that encodes for the subunits VirB2 and VirB5 that form the extracellular pilus structure. Besides, the encoding gene for VirB1 subunit predicted as a periplasmic lytic transglycosylase that plays a role in peptidoglycan alteration throughout T4SS biogenesis [39].

A recent study shows that in *Xcc* there are near to 12 proteins that interact with inner membrane associated ATPase VirD4, that is responsible for the recognition of substrates to be secreted [40]. These proteins share a C terminal domain termed XVIPCDs (Xanthomonas VirD4-interacting proteins conserved domains). These proteins are translocated into the target bacteria cell resulting in the dead of the receptor cells [41]. This bactericidal T4SS is knowing as X-T4SS and the effectors secreted by this nanomachine are termed X-Tfes (Xanthomonadales likeceeae t4SS effectors). Finally, a recent study reported that T6SS protect *Xcc* against the predatory amoeba Dyctiostelium [42].

3.2 Microbe -host interaction inside the susceptible host

Once the bacterium *Xcc* reaches the mesophilic tissue, after of epiphytic fitness and survival events mention before, must have to face the host defense response and parallel to express the pathogenicity factors;

3.2.1 T3SS the main pathogenicity determinant

The type 3 Secretion systems T3SS is the main protein secretion system widely studied in relationship to the pathogenicity. This secretion system is shared with several pathogenic bacteria ranging from animal to plants. This system is known as the "needle" and it works by delivering effector proteins directly to the target cells and modifying their behavior. Effectors from *Xcc* strains determine the host range. i.e. avirulence factors limit the specificity at the pathogen race/cultivar level by triggering immunity reactions in hosts with a related specific resistance gene. [43]. The effector delivered by the T3SS in *Xcc* belongs to the AvrBs3/PthA family. *Xcc* contains four PthA genes that encode transcription activator-like effector (TALE); of these four genes, pthA4 is responsible for the formation of citrus canker lesions. In citrus host the gene known as CsLOB is targeted by the TALE encoded by the *Xcc* gene pthA4; this gene was assessed in two susceptible host to *Xcc* infection, i.e., grape fruit and sweet orange [44]. CsLOB1-specific function still remains unclear; some previous studies suggest that CsLOB1 is involved in the regulation of development of lateral organ and metabolism of nitrogen and anthocyanin. Some plant hormones such as auxin, gibberellin, and cytokines also have proven to exert an effect on CsLOB1 gene [45]. Therefore, TALE have been shown to promote host cell transcriptional reprogramming as a virulence strategy [46].

4. Conclusions

The bacterium *Xcc* uses various adaptation and colonization strategies, it which are mainly aimed at guaranteeing its epiphytic survival, either by overcoming stress factors of biotic origin (predators, competitors, nutrient limitation) and abiotic origin (UV radiation, humidity and temperature variability). Because, this phase of epiphytic adaptation is crucial for the subsequent development of citric cancer symptoms in the susceptible host. Despite, these mechanism not having a direct effect on the health of the host, they become virulence factors, since its abolition avoid the subsequent development of the characteristic symptoms of citric cancer. Already inside the host, the bacterium uses as the main direct pathogenicity factor, the inoculation of effector proteins TALE, this effector is responsible for inducing cell hyperplasia, leading to rupture of the leaf epidermis and resulting in raised corky and spongy lesions surrounded by a water-soaked margin, the pathognomonic lesson of bacterial citrus canker.

Conflict of interest

The authors declare no conflict of interest.

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